LABORATORY MANUAL OF PHYSIOLOGY

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OF

PHYSIOLOGY

BY

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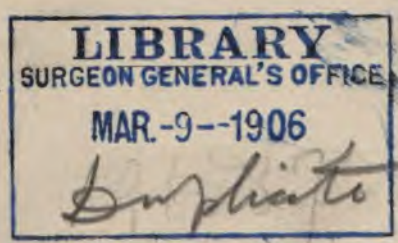

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PREFACE.

It has been the aim of the author, in compiling this little volume, to give an outline of experimental physiology for the guidance of students, in a brief and concise form, and sufficiently comprehensive in the subject-matter considered.

Descriptions of apparatus and illustrations have, to a large extent, been omitted, since these are more properly the function of the instructor and of the student himself. For the same reason, results and conclusions of experiments have been left to the student to work out for himself, the descriptions in the text being largely confined to methods of procedure involved in obtaining the results.

The arrangement of chapters and experiments can be modified to suit the individual instructor. The more difficult experiments may, if desired, be given as demonstrations. It has been the experience of the author that students work best in groups of two for the simpler experiments, and of four for the more complicated experiments.

The selection of the experiments presented has been made with a view to the needs of the medical student and to the practical application of the first-hand knowledge, obtained in the laboratory, to medical problems, later. At the same time, it must be borne in mind that one of the main benefits to be obtained from laboratory work is the training in methods of exact observation which the students receive.

The chapter on vision was prepared by Dr. Lee Masten Francis, to whom I take this opportunity to express my thanks.

Buffalo, N. Y., September, 1905.

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LABORATORY MANUAL OF PHYSIOLOGY.

CHAPTER I.

BIOLOGICAL INTRODUCTION.

Appliances.—An aquarium; Slides; Cover glasses; Test-tubes; Pipettes; Beakers; Microscope.

SINCE the basis of physiology as well as morphology is the cell, a few examples of the more common simple plant and animal cells are here presented for study, as a preparation to the observation of the physiologic phenomena accompanying the activity of the more highly differentiated cell-groups of the higher animals.

For this purpose, well-known representatives of the algæ, fungi, and protozoa have been chosen.

I. ALGÆ.

These are plant cells of the lowest order, consisting, either of single cells leading an individual existence, or of groups of cells attached end to end so as to form filaments or threads. A green coloring matter, chlorophyll, is common to the group.

1. Protococcus.—In the mud of shallow pools, ditches, and roof-gutters, a unicellular form, *Protococcus*, may commonly be found. This is seen, in the vegetative stage, as a spheroidal body, of small size, having an outer tough transparent envelope, composed, chiefly, of cellulose, and enclosing viscid granular protoplasm. Certain portions of the protoplasm contain the coloring matter, chlorophyll, which may be either green or red. These portions are known as chromatophores. The cell contains a distinct nucleus with a nucleolus. Reproduction takes place by the formation of so-called zoospores. These are of two kinds, macro- and micro-

spores. The former are produced by the division of the cell contents into two or four ovoid masses. These are set free through the resorption of the mother cell membrane, develop two cilia at opposite poles, and become free swimming forms. Later, a cell envelope is also formed. The microspores are smaller, more numerous, and are devoid of cell wall. Both forms, finally come to rest, lose their cilia, develop a thick cell wall, and again assume the vegetative condition.

Sunlight is essential to the growth of this plant as it is, in fact, to all chlorophyll plants. It is through the agency of the chlorophyll that carbon dioxide is broken down into carbon and oxygen for the constructive metabolism of the plant. This is a distinctive characteristic of the algæ and other chlorophyll plants as compared with the non-chlorophyll plants, such as the fungi.

Practicum.—Spread out some mud, containing protococcus, on a glass slide, dilute with water, and look for the plant with a low power of the microscope. Study with a high dry lens. Make out the following points· size; form; structure; zoospores. Stain with iodine. This kills the cell and may show the cilia.

Into two tubes, filled with and inverted over mercury, introduce some water rich in protococcus. From a carbon dioxide generator, introduce into each tube a few bubbles of the gas. Place one tube in the dark and the other in the light. After several hours, examine the tubes. Measure the gas in both. Place a small piece of KOH in each tube. Is the gas absorbed? In the tube that has been in the light, introduce a few drops of pyrogallic acid solution. Is any more gas absorbed? Explain.

Place some water, containing numbers of zoospores, on a slide; cover with a long cover slip and place under the microscope. With the substage mirror, cause a beam of bright sunlight to pass through the specimen. What is the effect on the movement of the spores? Now reduce the intensity of the light. What is the effect?

2. Spirogyra.—This form is commonly found, during the summer, in ponds and tanks, as floating masses of a light green color. These masses are found, on observation, to consist of long, fine

green threads. Each thread is made up of a number of cylindrical cells placed end to end. In each cell there are one or more spiral bands of a bright green color. These contain the chlorophyll of the plant and are called chromatophores. At intervals in the band, small, round bodies may be seen which contain proteid substance. Aside from the spiral bands and the thin layer of protoplasm lining the cellulose cell wall, the cell space is filled with so-called cell sap. This consists of water in which certain inorganic and organic substances are dissolved. The nucleus may be either central or peripheral. The growth of the plant is accomplished by cell division in the long axis, maintaining the thread formation.

Reproduction takes place through conjugation. This is a good example of sexual reproduction. Ordinarily this occurs in the following manner. Cells from two adjoining filaments send out protrusions toward each other. These meet and join, their adjoining membranes becoming absorbed so as to form one continuous tube. The cell contents of both contract, the one before the other, and the contents of one cell run through the tube into the other, nucleus uniting with nucleus, chromatophore with chromatophore. This is typical of sexual reproduction in both plants and animals. In this case, the new cell thus formed is called a zygospore. This, which is at first spherical in shape, without any distinct cell wall, increases in size by the imbibition of water, assumes the form of an ellipse and develops a hard envelope which is impermeable to water. In this condition the cell can withstand drying and considerable variations in temperature. When the plant again germinates, the cell wall is burst and the contents grow out into a new filament.

Practicum.—Observe, with unaided eye, a mass of spirogyra. Mount a few filaments and observe under first low and then high power of the microscope. Describe the structure. Make a drawing. Stain a specimen with carmine, after fixation in picric acid.

To observe the phenomena of reproduction, examine a fresh specimen that has been kept in the cold, over night.

Observe, from time to time, a specimen that has been kept in

distilled water. What is the effect on the life and growth of the plant?

Place a specimen in the dark for several hours. Examine for starch by treating with iodine. Result? Place some of this same material in bright sunlight for several minutes. Examine for starch again. Result?

II. Fungi.

Yeast.—(*Torula* or *Saccharomyces cerevisiæ*).—This plant, in common with the other fungi, differs from the algæ, which have been studied, in not containing any chlorophyll. The algæ, which contain this pigment, are able to obtain their nutrition from the inorganic constituents of their environment. The yeast, on the other hand, in which this pigment is absent, is dependent upon organic material for the processes of its metabolism.

Practicum.—Nutritive fluid for yeast (Pasteur's fluid).

Potassium phosphate	2.0	grams.
Calcium phosphate	0.2	"
Magnesium sulphate	0.2	"
Ammonium tartrate	10.0	"
Cane sugar	150.0	"
Water to	1000.0	"

(*a*) Put a small quantity of fresh baker's yeast into some of the above-described fluid and keep in a warm place. As soon as the culture becomes frothy and cloudy it is ready for examination.

(*b*) Place some of this mixture on a slide without a cover glass and examine with a low power of the microscope. Note size and arrangement of the cells. Place a cover slip on the specimen and examine with a high power. What is the mode of union of the cells? Describe the structure. Make a drawing.

(*c*) Stain a specimen with fuchsin. Treat another with iodine. Is there any starch present?

(*d*) Sow some yeast in Pasteur's fluid and place in the incubator for several hours. Place another specimen in the cold for the same length of time. Compare the growth in the two specimens.

(*e*) Take two other specimens, keeping one in the dark, the other in the light. Is there any change in growth?

(*f*) In a sterilized test-tube, place some yeast mixture and boil for several minutes. Replace the sterile cotton plug in the mouth of the tube and set to one side. Examine from time to time. Does growth continue?

(*g*) Taste some fresh yeast mixture. Taste again after the mixture has been in the incubator for twenty-four hours. To what is the difference in taste due?

(*h*) Grow some yeast in a tightly stoppered flask, connected, by means of a bent glass tube, with another flask containing a solution of calcium hydrate. What is the result? Explain.

III. Protozoa.

1. Amœba.—These simplest forms of animal life are distinguished from the simplest plant cells, not so much through their power of locomotion as through a difference in their processes of nutrition. The plant cells, which have been studied in the previous exercises, have been able to build up their living cell substance, protoplasm, a proteid body, out of non-proteid material. This the animal cells, of which the amœba is a type, are unable to do. In other words, the animal cells are dependent upon vegetable cells to manufacture their protein for them.

Fig. 1.—Amœba. Phases of amœboid movement.

Amœba are commonly found in stagnant pools, mud, and collections of water containing decaying vegetable matter. They are seen as minute jelly like masses, with a more or less granular interior and a clearer, more transparent peripheral portion (see Fig. 1).

Under certain conditions, they assume a spherical form. Generally, however, they are seen undergoing constant changes of form which, at times, may be very rapid. These changes consist in the protrusion, from various parts of the outer portion or ectosarc of the animal, of processes into which the more fluid interior portion flows. Such processes are called pseudopodia. By the formation of pseudopodia, the animal is enabled to move about. This, then, is a form of locomotion.

The amœba may contain one or more nuclei, usually only one. In some part of the outer portion, a large, clear space in the protoplasm is seen, alternately, to grow larger and smaller. This is the contractile vesicle or vacuole.

Practicum.—(*a*) Place a drop of amœbæ-containing water, on a slide. Cover with a supported cover slip. Examine with a low power. Having found an amœba, examine it with the high power. Make a drawing, indicating its main points of structure. Watch an active specimen and make outline drawings from time to time to show the change in form. Look for a specimen that is ingesting food. What is the process?

(*b*) Observe the effect of heat upon the movements by using the hot stage.

(*c*) In the same way that you have studied the amœba, study the white blood corpuscles of the frog and of man. To bring out the nuclei, treat with dilute acetic acid. The slides for the study of the living cells of man must be kept on a warm stage.

(*d*) Into the dorsal lymph sac of a frog, inject an aqueous mixture of carmine or a fine suspension of lamp-black. After fifteen minutes or a half hour, withdraw some lymph and examine for the ingestion of foreign bodies by the white cells.

2. Infusoria.—These are protozoa having bodies of a constant form and depending, for locomotion, upon flagella or cilia. The flagella may be single or double and may be attached to one or both poles of the organism. One of the most common found in aquaria is Euglena viridis, having a long whip-like flagellum at one end, by means of which it swims rapidly through the water.

The lowest forms of flagellata are the trypanosomata, a parasitic type of which is shown in Fig. 2. These forms have lately become of medical interest because of their apparent causal relation to certain tropical diseases.

Common examples of the ciliated protozoa that are found in

FIG. 2.—Frog's Blood. *A*, Trypanosome; *B*, eosinophile. (Williams.)

ponds and stagnant pools are Paramecium and Vorticella (see Figs. 3 and 4).

The former is a free swimming form, rather oval and somewhat flattened in shape, with a mouth-like aperture at one side, leading into a short stomach-like pouch. The animal is covered with cilia which are longer in the mouth region and at the posterior end. A nucleus can be distinguished, as well as two contracting vesicles, one at either end of the animal.

The Vorticellæ are seen as solitary individuals, consisting of an oval body mounted on a long stalk which is attached to some foreign body. At the free end, a flattened disc surrounded by cilia is seen. At one side of this is the aperture known as the mouth.

On closer examination, it is seen that the band of cilia does not

form a complete circle, but rather a spiral, which passes down the tube leading from the mouth and known as the esophagus. There is a sausage-shaped or spiral nucleus and a contracting vacuole. The stalk may be seen under the high power to contain a core of contractile protoplasm.

Practicum.—(*a*) Place a little powdered carmine in the water containing vorticellæ. Examine under the microscope. What be-

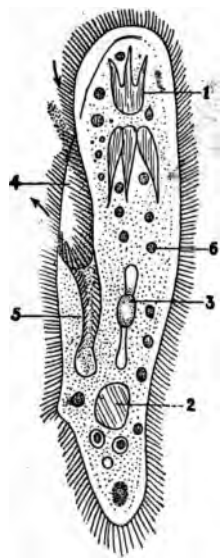

FIG. 3.—Paramecium. 1 and 2, Contracting vacuoles; 3, nucleus; 4, mouth; 5, stomach; 6, food particles.

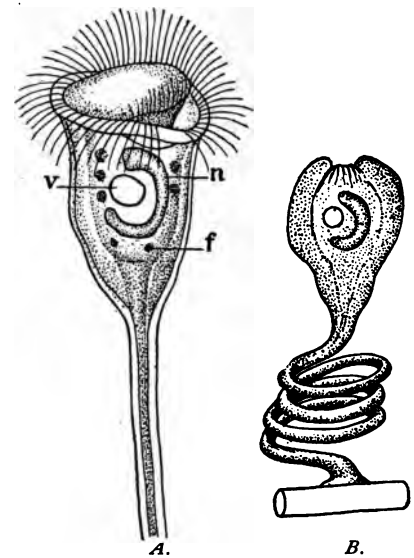

FIG. 4.—Vorticella. *A*, Open, stalk extended; *B*, closed, stalk coiled. *n*, Nucleus; *v*, vacuole; *f*, food particles.

comes of the carmine? Note the motion of the cilia. Follow the movement of the food vacuoles. Note the movements of the animal as a whole. What is the function of the stalk? If opportunity offers, observe the process of multiplication.

(*b*) *Effect of various gases upon the activity of protozoa. Carbon dioxide.* Arrange a deep-celled slide in such a way that a

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forks, the time-marking lever may be caused to vibrate any desired number of times per second.

The number of interruptions is varied until a frequency is obtained which shuts off the light in such a manner that the cilia appear stationary. This frequency corresponds to the rate of vibration of the cilia.

3. Pith a Frog.—This, ordinarily, means destroying the brain, and is accomplished in the following manner: Hold the frog in the left hand, securing the head between the index and middle fingers, with the thumb over the back. Bend the head forward so as to place the occipito-atlantal ligament on the stretch and expose the articulation between the skull and the vertebral column. With fine scalpel or fine pointed scissors, make an incision through the neuraxis at this point. Run a blunt-pointed seeker through this opening into the cranial cavity and destroy the brain. If required, the cord may be broken up in a similar manner.

(*a*) With heavy scissors, cut off the lower jaw of the frog. Place the frog on its back; wash off the mucous membrane of the roof of the mouth with normal salt solution; remove the excess with filter paper; place a small piece of cork on the mucous membrane near the apex of the jaw and watch its movement. Time the movement of the cork for a certain distance.

(*b*) Repeat the experiment after having bathed the mucous membrane with warm salt solution (36° C.).

(*c*) Repeat again after bathing with cold salt solution (5–10° C.). What is the effect of temperature upon ciliary motion?

(*d*) The above experiment may be repeated, using the apparatus shown in Fig. 5. For this purpose, dissect out a piece of the frog's œsophagus; pin it, outer side down, upon a cork board; adjust the weight (W) and the lever (P) so that the rate of ciliary movement may be indicated on the graduated arc (S).

(*e*) Using the same apparatus, try the effect of various weights upon the ciliary motion, beginning with five grams and increasing the weight until the cilia are no longer able to move it.

(*f*) Repeat this experiment with a fresh preparation, tilting the

cork board at an angle of about thirty-five degrees. Flat weights will be necessary on account of the incline. Measure the height of the upper edge of the preparation from the lower edge. Observe the time taken for the passage of a given weight through this dis-

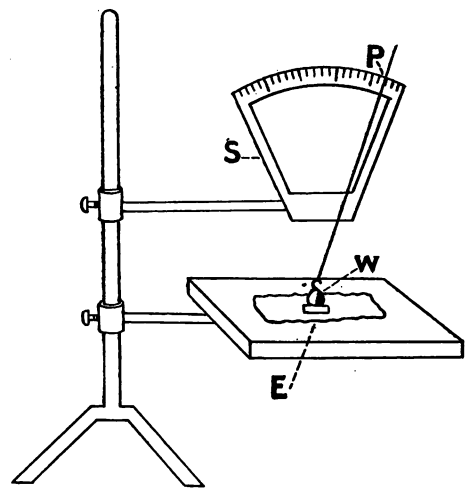

FIG. 5.—To Show Ciliary Motion. (According to Kronecker.) *E*, Portion of frog's œsophagus; *W*, cork with weight; *P*, pointer; *S*, scale.

tance. Estimate the work by the cilia, according to the following formula: W, work done; G, weight in milligrams; H, height in millimetres. W, then, would equal G multiplied by H. What is the work done per second? Per minuute?

4. Effect of CO_2 on Ciliary Motion.—(*a*) Fill a small bell jar with water and invert over water. Introduce a tube from a CO_2 generator and fill the jar with the gas. Pin an œsophagus preparation to a cork board and place on a glass plate greased with vaseline. Place a cork with weight upon the preparation. Quickly adjust the gas containing jar over the ciliary preparation. Observe the rate of ciliary motion, as before. What is the effect of the gas?

(*b*) Repeat the above experiment, placing a piece of absorbent cotton, saturated with chloroform, under the jar. Effect?

(*c*) Repeat, using ether. Ammonia. Other gases.

5. Keep a piece of frog's œsophagus, moistened with normal saline, for several days. From time to time scrape off a bit of the epithelium and examine under the microscope for active cilia.

How long do the cilia survive after the death of the frog? What is the function of ciliated epithelium? Where is it found in man?

CHAPTER II.

MUSCLE-NERVE.

Appliances.—Revolving drum; Myograph (heavy); Light muscle lever; Inductorium; Moist chamber; Platinum electrodes; Non-polarizable electrodes; Rheostat; Rheocord; Rheonome; DuBois keys (2); Pohl's commutator; Glass plate for holding nerve; Cut-out key; Capillary electrometer; Current interrupters; Signal magnets; 4 dry cells; Light copper wire; Various chemical reagents (see text below).

I. Study of Electrical Apparatus.

1. The Galvanic Current.—The fundamental experiment upon which the principles of this form of electricity have been based was unwittingly performed by Galvani in 1786. Galvani noted that some frog's shanks, which had been hung by copper wires from an iron railing and which were swinging to and fro, twitched whenever they came in contact with the railing. It is true that Galvani misinterpreted the results of this particular experiment, ascribing the phenomena to the development of an electric current within the tissues themselves. That there are tissue currents, he did show by later experiments.

It was demonstrated, however, by Volta, a contemporary, that Galvani's initial experiment was due to the production of a current through completing the circuit between two metals of a different potential. This constant flow of current between two substances of different potential has been called galvanic electricity, in contradistinction to that form consisting of a single discharge or a series of discharges from one body to another and where the current is but of an instant's duration. The latter is known as static electricity.

The galvanic cell, as usually constructed, consists of two metals,

such as zinc and copper, partly immersed in a dilute acid or solution of a salt. It is now considered that the current is not due so much to the difference of potential of the metals as it is to the dissociation of the solution into its so-called ions. These are supposed to be charged with positive and negative electricity. Thus, if H_2SO_4 be used as the electrolyte, the H group represents ions which are electropositive, while the SO_4 group is electronegative. These ions, charged with positive electricity, move toward the negative pole, in this case, zinc, while those charged with negative electricity, move toward the positive pole, in this case, copper. The former are termed kations; the latter are known as anions. When the two metals are connected by a conductor, there is a flow of current from the place of highest intensity, the anode, to that of lower intensity, the cathode. This may be compared to the flow of water from areas of high pressure to areas of lower pressure. The energy upon which the flow depends is known as the electromotive force (E.M.F.). Its unit of measurement is the VOLT.

The flow of the current meets with more or less resistance which has to be overcome. This resistance is inversely proportional to the length and thickness of the conducting substance. It also varies with the nature of the substance itself, irrespective of its length and thickness. There is resistance in the cell itself. This is known as "internal" resistance to distinguish it from that in the wire which is known as external resistance.

Where the external resistance is high, as in passage through tissues, the internal resistance of the cell is a negligible quantity.

2. Ohm's Law.—The relations between electromotive force, resistance, and intensity of current, are formulated as Ohm's law. If I represents intensity of current, E.M.F., electromotive force, and R, resistance, then $I = \frac{R}{E.M.F.}$

The unit of measurement for the strength of current is the Ampere. This represents the quantity of electricity passing over a given cross-section of conductor in a given time. That amount of electricity which will deposit 0.001118 grams of silver per second

is known as a Coulomb. The Ampere equals one Coulomb passing a given point per second.

The unit of resistance is the Ohm. The standard ohm is the resistance offered by a column of mercury 1.06 meters high with a cross-section of one square millimetre. This is, approximately, equivalent to the resistance of a copper wire, $\frac{1}{20}$ inch in diameter and 250 feet long.

The VOLT is the unit of E.M.F. It is the E.M.F. required to send one ampere of current through one ohm of resistance in one second.

3. Regulating the Amount of Current.—According to Ohm's law, it is apparent that the current may be regulated in one of two ways or by a combination of the two; *i.e.*, by changing the E.M.F. (voltage), or by changing the resistance. The resistance remaining the same, an increase in voltage will give an increase in current. With the voltage remaining the same, an increase in resistance will give a decrease in current.

In the laboratory this is accomplished in two ways, either by the manner in which the battery cells are connected up or through interposing resistance in the circuit by means of rheostats or rheocords.

4. Arrangement of Battery Cells.—If plus pole be joined to plus pole, the cells are said to be connected in parallel. If minus pole be connected to plus pole, the cells are said to be joined in series. In the former case, the amperage is increased with but little change in the voltage. With connection in series, the voltage is increased with but little change in amperage.

An increase in both voltage and amperage, at the same time, may be obtained by joining the cells in "multiple." This is accomplished by joining groups of cells in series and then arranging these groups in parallel.

Resistance is obtained by the use of the resistance box, consisting of coils of German-silver wire which may be short-circuited by means of metal plugs interposed between the coils; or by the use of the rheocord, consisting of a long loop of the same wire which

may be short-circuited anywhere along its length by means of a sliding metal rider.

5. Induced Currents.—If a conductor through which a current is flowing is brought near to another and parallel conductor, an instantaneous current is induced in the latter and opposite in direction to that of the former. When the first conductor is again removed from the vicinity of the second, a current is again induced in the second, but now in the same direction as that in the first. The same effect is produced by making and breaking the circuit in the first wire or by alternately bringing a conductor into and away from the vicinity of a magnet.

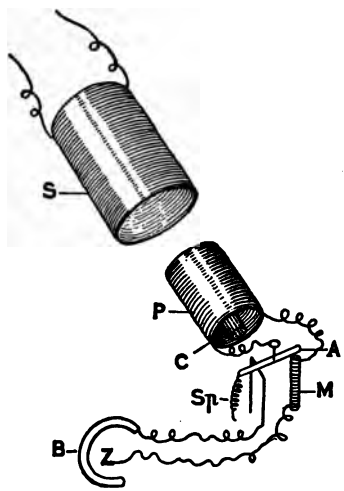

FIG. 6.—Diagrammatic Drawing of Inductorium. *P*, Primary coil; *S*, secondary coil; *C*, core; *B*, battery; *M*, magnet of interrupter; *A*, armature of interrupter; *Sp*, spring of interrupter.

The Inductorium, used in the physiological laboratory, is based on these principles (see Fig. 6). It consists of a primary coil of heavier wire of few windings (P), surrounding a movable core of iron wire, a secondary coil (S) of finer wire and many more windings, not connected with the primary coil, and an automatic interrupter which may or may not be placed in circuit with the primary coil.

II. PRACTICUM.

Take two hollow spools, one considerably larger than the other, winding the small one with heavy insulated copper wire and the large one with fine insulated copper wire. The small coil is the primary and is to be connected with the battery. The large coil is the secondary and is to be connected with the galvanometer.

MUSCLE-NERVE.

1. Place the primary inside of the secondary. By means of a key interposed in the primary circuit (*a*) make the current. Is there any movement of the galvanometer needle? What is the direction and degree of this movement? (*b*) Break the primary circuit. What is the movement of the galvanometer needle? Does the needle remain in the position which it assumes upon either the make or break of the primary circuit? (*c*) Repeat (*a*) and (*b*), moving the primary farther and farther from the secondary. Is there any difference in the excursion of the galvanometer needle? Is there any difference in the degree of excursion of the needle at make, as compared with break of the primary circuit?

2. (*a*) With the key of the primary circuit closed, suddenly withdraw the primary coil from the secondary. What is the effect on the position of the galvanometer needle? (*b*) Now suddenly approach the primary toward the secondary and note the effect on the galvanometer.

3. (*a*) With the primary placed to one side, introduce a permanent magnet rod into the secondary. What is the effect on the galvanometer? (*b*) Withdraw the magnet. What is the result?

4. With a simple rheocord in the primary circuit, suddenly increase or decrease the resistance by quickly moving the slider back and forth. Is any current induced in the secondary through changing the intensity of the primary current?

5. Apply the electrodes from the secondary coil of an inductorium to the tip of your tongue. Open and close the primary circuit with the primary some distance removed from the secondary. Repeat, gradually moving the secondary toward the primary until the shocks produced are too strong for comfort. Which shock is first detected by the tongue? Why should the break shock be stronger than the make? What is the possibility of the production of induced currents in the coils of the primary itself? What would be their effect on the make as compared with the break currents? These currents are known as make-extra and break-extra currents.

III. Interrupters.

For interrupting the primary circuit, any one of a number of devices may be used. Where very rapid succession of shocks is desired, as in the production of the so-called tetanizing current, the Neef's hammer is used as shown in Fig. 6. Where known fre-

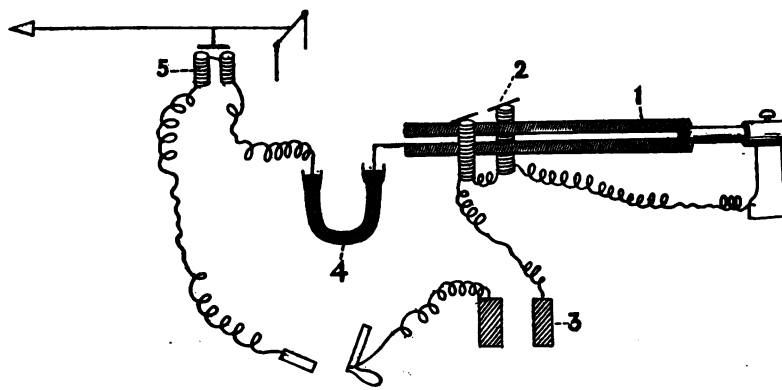

Fig. 7.—Tuning-fork Interrupter. (According to Kronecker.) 1, Tuning fork; 2, electro-magnet, alternately made and broken; 3, battery; 4, mercury contact; 5, time marker.

quency of interruption is necessary, a Bowditch clock or similar arrangement may be used for low frequencies and metronomes and electrically maintained tuning forks, for higher frequencies (see Fig. 7).

IV. Dissection of the Frog's Thigh and Leg.

With a preserved frog or a fresh one that has had brain and cord destroyed, carefully dissect and identify the muscles of the thigh and leg (see Fig. 8).

1. Gastrocnemius-sciatic Preparation.—Pith a frog. Remove the skin from one thigh. Make a circular incision through the skin at the knee and another at the lower end of the leg. Slide this up as far as the knee. This is to be slipped back, later, over the muscle to keep it from drying. Separate the gastrocnemius

muscle from the tibia. Cut the tibia through, just below the knee, being careful to avoid injury to the nerve where it enters the muscle on its upper and dorsal aspect. Tie a thread about the

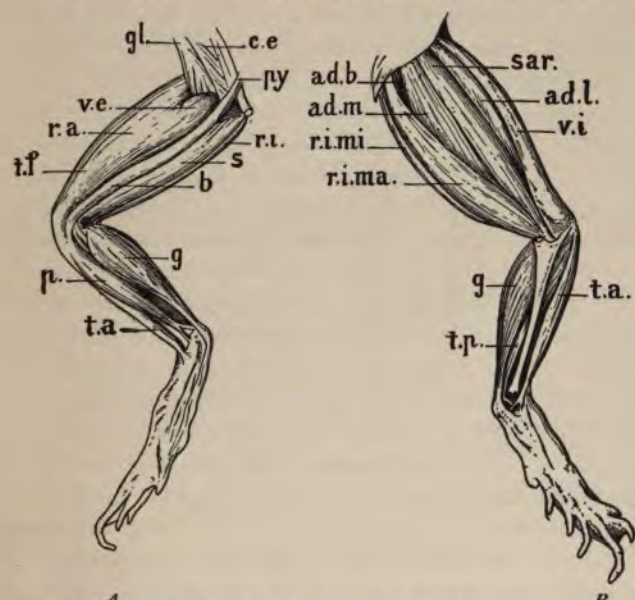

FIG. 8.—Muscles of Frog's Thigh and Leg. *A*, Dorso-lateral view. *gl*, Gluteus; *c.e*, coccygeus; *py*, pyriformis; *v.e*, vastus externus; *r.a*, rectus anterior; *t.f*, triceps femoris; *r.i*, rectus internus; *s*, semimembranosus; *b*, biceps; *g*, gastrocnemius; *p*, peroneus; *t.a*, tibialis anticus. *B*, Ventro-lateral view. *sar*, Sartorius; *ad.l*, adductor longus; *ad.b*, adductor brevis; *ad.m*, adductor magnus; *r.i.mi* and *r.i.ma*, rectus internus minor and major (or gracilis); *g*, gastrocnemius; *t.a*, tibialis anticus; *t.p*, tibialis posticus.

tendo Achillis, just above the sesamoid cartilage. Cut the tendon below the ligature.

On the dorsal side of the thigh, carefully separate the gluteus maximus muscle and biceps from the semimembranosus, using the fingers for the purpose. This exposes the sciatic nerve. Carefully separate it from the surrounding muscles, avoiding pulling and stretching as much as possible. It is well to handle the nerve with

a glass hook. Cut the thigh muscles near their insertions about the knee, being careful not to cut the nerve. Cut the muscles, also, near the pelvic articulation of the femur. Remove all the abdominal viscera, thus exposing the nerve in the lumbo-sacral plexus. Cut through the vertebral column just above the last two vertebræ which join the urostyle. Remove the muscles on each side of the urostyle. Cut through the junction of the last vertebra with the urostyle, and, using the two vertebræ as a handle, lift the nerve from its bed from above downward, cutting its branches and carefully freeing it from the groove over the femoro-pelvic articulation. Cut through the femur just below its articulation with the pelvis and the preparation is complete (see Fig. 9). The femur may be clamped in the muscle clamp and the thread about the tendo Achillis, to the myograph lever. Both nerve and muscle should be kept moist with physiological salt solution.

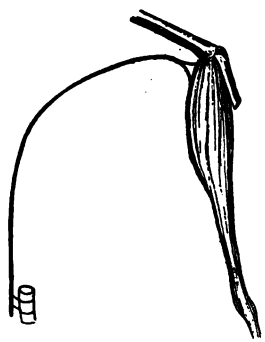

FIG. 9.— Gastrocnemius-sciatic Preparation.

2. Double Semimembranosus-gracilis Preparation.—Dissect out the semimembranosus and gracilis muscles of both sides, using the same precautions as in the previous preparations. Both femurs should be disarticulated at the hips and the pelvis cut through transversely, thus leaving the two muscles joined by a thin piece of bone. This may be secured in the femur clamp.

3. The Sartorius.—This corresponds to the muscle of the same name in human anatomy. It is a long, thin muscle having its origin from the symphysis pubis and its insertion into the capsule of the knee, fascia of the leg, and tibia. This muscle is to be used where parallel fibres are desired.

V. ELASTICITY OF MUSCLE.

Make a gastrocnemius-muscle preparation, clamp the femur in the femur clamp, attach the tendon to the lever of the myograph,

and adjust the writing point against the smoked paper of a drum. The lever should be nearly tangent to the surface of the drum and the drum should revolve away from the point of the lever. Arrange the drum so that it may be revolved by hand.

1. To Show the Elasticity of a Rubber Band.—Attach a rubber band to the femur clamp and myograph lever and adjust for writing on the smoked drum. Carefully place a 10-gram weight in the pan attached to the lever. Move the drum slightly to record the amount of stretching. Remove the weight, allow the lever to return through the elasticity of the band and revolve the drum again slightly. Repeat this with 20 grams, with 30 grams, with 40 grams, with 50 grams. Does the lever return each time to its original position?

2. To Show the Elasticity of Muscle.—Repeat the above experiment, using the muscle already prepared. How does the elasticity of the muscle compare with that of the elastic band? If the elasticity is not perfect for the amount of stretching force employed, are there any factors of error in the apparatus and method that may, in part at least, account for the results obtained?

3. To Show the Tensile Strength of Muscle.—With the same preparation used in the previous experiment, carefully add weights to the pan of the lever until something gives way. Which breaks first, the muscle or the tendon? Prepare a fresh muscle and repeat, inserting needle electrodes into the muscle and stimulating with a tetanizing current from an inductorium for each addition of weight. How much will the muscle lift?

4. Hooke's Law.—This is embodied in the statement that the power of any spring is in direct proportion to the tension under which it is placed. Does the muscle in experiment (*b*) respond to Hooke's law where small weights are used? Recent experiments by Professor Haycraft, with improved apparatus from which all sources of error have been eliminated, show that, within physiological limits, all the simple tissues of the body follow this law.

VI. Irritability of Nerve and Muscle to Varius Stimuli.

Nerve and muscle tissue have in common the properties of irritability. The muscle has, in addition, the power of contractility. In the following experiments the stimulating agents will be applied to the nerve and the contraction of the muscle will be used as an indicator of the efficacy of the stimulus. The stimulating agents may be grouped as mechanical, thermal, chemical, and electrical.

1. Mechanical Stimuli.—Make a sciatic-gastrocnemius preparation. Place the nerve on a glass plate and keep both muscle and nerve moist with physiological salt solution. (*a*) Cut the nerve near its origin from the cord with sharp scissors. Does the muscle contract? Tap the nerve with a scalpel handle.

(*b*) Cover the nerve with moistened filter paper, place a thin sheet of cork over this; place a small beaker very carefully on the cork and slowly pour mercury through a glass tube of small calibre into the beaker. Does the muscle contract? Which is the more efficacious, a stimulating force gradually applied or one suddenly applied?

2. Thermal Stimuli.—Heat a needle or a copper wire in the flame of a Bunsen burner to a red heat. Touch the nerve with it. Result? Will this piece of nerve respond again to stimulation? Explain.

3. Chemical Stimuli.—Make a fresh muscle-nerve preparation. Cut the nerve high up. Place on a glass plate, allowing the end of the nerve to hang over the edge. Use a watch glass or other small vessel for containing the reagents to be used. This is brought up to the nerve until its end dips into the contained reagent. That portion of the nerve used is usually destroyed by the chemical so that it is necessary to cut off the end of the nerve after each test. In this manner, try the effect of the following reagents.

(*a*) Concentrated solution of sodium chloride.

(*b*) Concentrated solution of sodium or magnesium sulphate.

(*c*) Fifty-per-cent alcohol.
(*d*) Glacial acetic acid.
(*e*) Five-per-cent sulphuric acid.
(*f*) Ammonia.
(*g*) Zinc chloride.
(*h*) Allow the nerve to dry.

4. Electrical Stimuli.—For this purpose, induced currents from an inductorium will be used. The effect of the constant current will be taken up later.

Make a muscle-nerve preparation, arranging for recording upon the smoked drum. Set up the inductorium for single shocks, interposing a short-circuiting key in the primary circuit for this purpose. Remove the secondary coil, as far as the instrument will allow, from the primary. In those instruments where the secondary is movable to form angles of varying degrees with the primary, the intensity of the secondary currents may be diminished by increasing the angle between the primary and secondary wires.

Apply the electrodes from the secondary coil to the nerve or to the muscle directly. Close the primary circuit. Result? Record on drum. Rotate the drum slightly and break the primary circuit. Result? Move the secondary nearer to the primary and repeat the make and break as before, recording results. Repeat again and again, gradually moving the secondary toward the primary until the muscle ceases to increase in the height of its contraction. Which is the more efficacious, the make or the break shock from the inductorium, and why?

VII. Period of Latent Stimulation and Form of the Single Twitch.

Arrange a drum to be rapidly spun by hand. With a little practice, this method gives as good results as the pendulum or spring myograph. Arrange a muscle-nerve preparation with point of writing lever touching the smoked surface of the drum. Arrange the writing points of two signal magnets (*a*) and (*b*), exactly under the writing point of the muscle lever. Place signal magnet (*a*) in

circuit with the primary. Place signal magnet (*b*) in circuit with the tuning fork giving 100 interruptions per second. Introduce a short-circuiting key in each circuit. Place the nerve of the muscle preparation on the electrodes from the secondary coil of the inductorium.

The three levers, the one attached to the muscle, that of signal magnet (*a*), which is in circuit with the current stimulating the nerve, and that of signal magnet (*b*), which is in circuit with the tuning fork, will then begin their tracings directly under each other, so that exact time comparisons may be made.

Close the short-circuiting keys in both circuits. Start the drum to spinning rapidly. When the drum has reached the height of its speed, open both keys. Close the tuning fork key immediately. Stop the drum. There will be three tracings to interpret. The signal magnet (*a*) marks the exact instant that the nerve was stimulated. The muscle lever marks the period of contraction of the muscle. The tuning-fork lever marks the time relations.

Does the muscle twitch begin exactly at the moment of stimulation? If not, how much time elapses between the application of the stimulus and the onset of contraction?

What is the form of the single-twitch curve? How does the period of contraction compare with that of relaxation?

VIII. The Velocity of the Nerve Impulse.

With the same arrangement of apparatus as in the previous experiment, ascertain the period of latent stimulation when the stimulating electrodes are on the nerve near the muscle; when the electrodes are on the nerve some distance from the muscle. Measure the length of nerve between the two points of stimulation. Estimate the difference in time of latent stimulation. This difference will be equivalent to the time that it takes the nerve impulse to travel over the length of nerve measured. From this the velocity per second can be easily determined.

IX. Direct Irritability of Muscle; Action of Curare.

Inject into the dorsal lymph sac of a frog a few drops, about one-half cubic centimetre of a one-per-cent solution of curare. First, however, dissect out one sciatic nerve, passing a ligature under it and tying it tightly about the thigh. All of the frog except that portion below the ligature will come under the influence of the drug (see Fig. 10). In fifteen or twenty minutes the drug action should be complete. Make two sciatic-gastrocnemius preparations, one of the curarized side and one of the non-curarized side. Set up the inductorium for tetanizing currents. Attach muscles to myograph levers for recording. Stimulate nerve of curarized side. Result? Stimulate the nerve of the non-curarized side. Result? Stimulate the muscle of the curarized side directly. Result?

Fig. 10. — Curare Experiment. *l*, Ligature around thigh; *s*, sciatic nerve, not included in ligature. Shaded area, affected by curare; non-shaded area, unaffected by curare.

What is the action of curare, as deduced from these observations? Is the muscle fibre itself directly irritable, aside from its nerve?

In the experiment under *electrical stimulation*, it was demonstrated that, up to a certain point, the height of a single muscle twitch is in direct proportion to the strength of the stimulus; *i.e.*, a minimal stimulus is accompanied by a minimal contraction and a maximal stimulus by a maximal contraction. This will be compared, later, with the action of heart muscle under similar circumstances.

X. Influence of Load on Muscle Twitch.

When the weight is continuously supported by the muscle, both when at rest and when contracting, the muscle is said to be loaded.

When the weight is supported by the muscle, only during the period of contraction, the muscle is said to be after-loaded. Compare the muscle curves obtained with load and after-load, using first small weights and then heavier and heavier weights.

XI. Effect of Temperature upon the Muscle Curve.

For the study of the effects of changes of temperature upon the muscle curve, the muscle warmer of Porter is very convenient. Where this is not at hand, the same results may be obtained by the use of a bath of physiological salt solution, which may be cooled or heated to the desired temperature and in which the muscle may be immersed, except for the short period required for stimulating and recording contractions.

1. Place the muscle in a small test tube surrounded by an ice pack until the temperature of the interior of the tube has fallen to zero or less, *i.e.*, until the freezing point has been reached. Remove the muscle and record a single twitch. Label the tracing.
2. Warm the muscle to 5° Centigrade and again record a twitch.
3. Warm the muscle to 10° C. and again record.
4. Warm to 15° C. and record again.
5. Warm to 20° C. and repeat record.
6. Warm to 30° C. and record again.
7. Warm to 40° C. and record again.
8. Bathe the muscle with salt solution heated to 45° C. and note result. The muscle passes into *rigor*.

Compare the curves obtained at the different temperatures and tabulate your results. What is the effect on the height of contraction? On the time of the contraction phase? On the relaxation period?

XII. Influence of Fatigue on the Form of the Single Muscle Twitch.

1. Set up the apparatus for automatic stimulation of the muscle or nerve as shown in Fig. 11. Make a sciatic-gastrocnemius preparation. Arrange the inductorium for maximal stimulation.

Place the drum contact arrangement in circuit with the primary. Adjust the myograph lever to the smoked surface of the drum. Allow the drum to revolve at its greatest speed. According to the arrangement shown in Fig. 11, six contractions will occur during

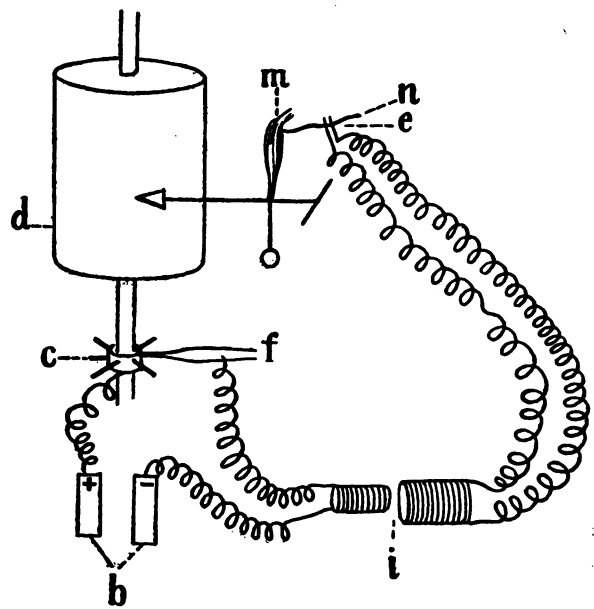

FIG. 11.—Drum Arrangement for Muscle-nerve Stimulation. *d*, Drum; *m*, muscle; *n*, nerve; *e*, electrodes; *b*, battery; *i*, inductorium; *c*, collar with contacts which, as drum revolves, make and break primary circuit with *f*, metal strip.

every revolution of the drum. Every sixth contraction will be recorded at the same place on the drum. In this way a number of superimposed contractions are recorded.

Allow the drum to revolve until the muscle ceases to respond by a contraction. What changes does the contraction curve undergo with the progression of fatigue?

Compare the fatigue curve with the temperature curves obtained in the previous experiments.

2. Make a fresh muscle-nerve preparation. Let the drum re-

volve slowly. Attach the muscle to the myograph lever. After-load with a 10-gram weight. Adjust lever to drum. Stimulate the nerve once a second with a submaximal induction shock.

A fatigue record formed of single twitches, written one after the other, will thus be obtained.

3. Repeat the above fatigue experiment with a loaded muscle.

XIII. Volume of Contracting Muscle.

In answer to the question, does the volume of the muscle change during contraction, some such device as that shown in Fig. 12 may be used. The muscle should be put in a container filled with physiological salt solution, the ends of the muscle being attached to electrodes from the secondary coil of an inductorium arranged for tetanizing shocks. The mouth of the container is closed with a tightly fitting cork, perforated for the passage of a fine glass tube in which the water from the container rises.

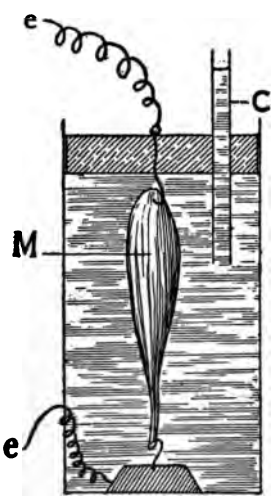

Fig. 12.—To Determine Volume of Contracting Muscle. *M*, Muscle; *e e*, electrodes; *c*, capillary tube.

Stimulate the muscle with tetanizing induction shocks and observe the level of the fluid in the capillary tube connected with the muscle container. Does the fluid rise or fall? Does the volume of the muscle change during contraction?

XIV. Summation of Stimuli.

Arrange inductorium with secondary coil removed from primary until single break shocks are just insufficient to cause a muscle twitch. Let the muscle rest for several minutes. Now stimulate the nerve every four or five seconds. Does the muscle finally contract? Explain.

XV. Summation of Contractions and Genesis of Tetanus.

Make a semimembranosus-gracilis muscle preparation. Clamp bony attachment in the muscle clamp. Attach the other end to the muscle lever. Connect muscle up for direct stimulation with the secondary coil of an inductorium, arranged for single shocks. In order that the stimuli may be of equal intensity, it is well to use

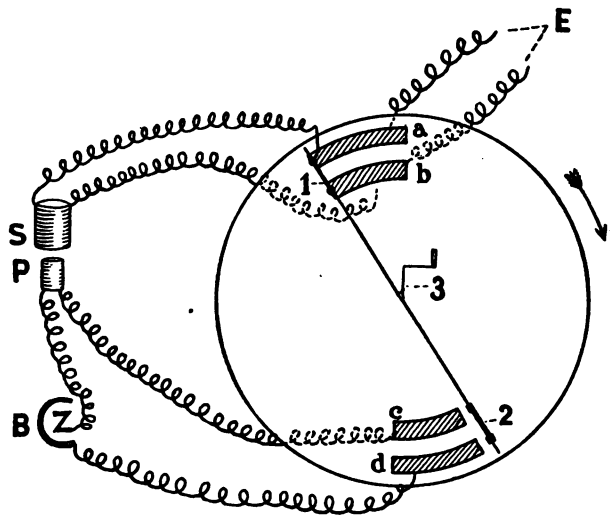

Fig. 13.—Cut-out Key. *B*, Battery; *P*, primary coil of inductorium; *S*, secondary coil; *a*, *b*, and *c*, *d*, metal strips; 1 and 2, metal clip contacts to complete circuit between *a*, *b*, and *c*, *d*; 3, crank to revolve clip contacts 1 and 2. Contacts revolved in direction of arrow. Contact at *a*, *b*, is made an instant before that at *c*, *d*. The secondary circuit is therefore short-circuited before the primary is made. The secondary is opened before the primary is broken. Hence the make shock is cut out. Connect *S* with *c*, *d*, and *P* with *a*, *b*, to cut out break shock.

some form of cut-out key, so that either the make or break shock may be eliminated (see Fig. 13).

1. Arrange key to give only break stimuli. Stimulate muscle with one break shock. Note form of contraction curve and height of contraction. The drum should be revolving at medium high speed.

2. Allow the muscle to rest for a moment. Stimulate again, and,

before the relaxation of the muscle is complete, send in a second stimulus. Repeat, decreasing the interval between stimuli until the two contractions merge to form one. This is known as the summation of contractions. How does the height of the two summed-up contractions compare with that of the single twitch? Where the second contraction begins during the relaxation phase of the first, what determines the height of the second?

3. Place the primary of the inductorium in circuit with a metronome or vibrating rod interrupter. Stimulate the muscle four times per second; five times per second; six times per second; eight times per second; ten times per second; twelve times per second; fifteen times per second; twenty times per second. Allow the muscle sufficient rest between the series of stimuli in order to avoid fatigue.

How do the contraction curves change as the frequency of stimulation is increased? How many stimuli per second are needed to cause the individual curves to merge into one apparently continuous curve? The latter condition is called *tetanus*. Where the individual twitches are still visible, the condition is one of incomplete tetanus.

4. That this condition of complete contraction is apparent rather than real may be shown by stimulating the nerve of a muscle and connecting the muscle itself with a capillary electrometer which will show a series of action currents corresponding to a series of single twitches. The capillary electrometer and the action current of muscle will be described later.

5. Influence of Temperature on the Production of Tetanus.—Repeat the previous experiments with a muscle cooled to 5° C.; with a muscle warmed to 35° C. How does the frequency of stimulation necessary to produce tetanus in the cold muscle and in the warm muscle compare with that required for the muscle at room temperature?

6. Influence of Fatigue on the Production of Tetanus.—After the muscle has become tired from repeated stimulation, repeat the foregoing experiments. Result? Explain.

XVI. To Determine Actual Shortening of a Muscle in Contraction.

Divide distance of the writing point of the muscle lever from the axis of the lever by the distance of the muscle attachment from the axis. Then divide the height of the recorded curve by this factor. The result is the actual shortening of the muscle during contraction.

XVII. To Determine the Work Done by the Muscle during any particular contraction, multiply the actual shortening by the load. Thus, if the actual shortening or height of contraction is 5 millimetres and the load is 10 grams, then the work done would be 50 gram-millimetres.

1. On a drum revolved by hand, record the heights of contraction of a gastrocnemius which is receiving submaximal stimuli. After-load the muscle successively with 10, 20, 30, 40, 50, 70, 100, 150, 200, 250, 300, 350, 400, 450, and 500 grams.

2. Estimate the actual work done according to the formulæ given above. Plot a curve, marking, on the abscissa, intervals to represent 50-gram weights; on the ordinates, intervals to represent gram-millimetres. What conclusions can you draw from the data thus plotted?

XVIII. Fatigue of Human Voluntary Muscle.

Ergography.—The contraction of voluntary muscle is normally brought about through nerve impulses originating in nerve cells. The nerve cell, as will be shown later, has a certain rhythmic activity, sending out from 6 to 10 impulses per second. This seems to be sufficient to keep the muscle in a state of tetanus. The shortest voluntary muscle contraction, then, brought about through the discharge of nerve impulses from nerve cells, is a tetanus. The single twitch occurs only under abnormal circumstances, or through artificial stimulation of the nerve or muscle directly. Any stimulation of nerve cells, sufficient to cause a discharge of nerve impulses, will produce a tetanus in the muscle receiving the impulses.

For recording the contractions of human voluntary muscle, either the ergograph of Mosso or that of Porter may be used. With the former, the flexion of the middle finger is recorded; with the latter the contractions of the abductor indicis.

1. If the Mosso instrument is used, place the forearm and fingers in the securing attachments of the apparatus and weight the middle finger with one or two kilograms. Contract the muscles, voluntarily, once every two seconds, keeping time to the beat of a metronome, until you are no longer able to bring about a contraction in this way. The contractions should be recorded upon a slowly revolving drum. Now stimulate the flexor muscles directly with electrodes placed over the forearm, using the same frequency of stimulation as before, one every two seconds. Does the muscle respond to direct stimulation after fatigue has been induced to volitional impulses?

2. Repeat with a new subject, reversing the procedure. In other words, stimulate the muscle artificially until it no longer responds and then attempt to flex the finger voluntarily until complete fatigue is obtained.

3. With a fresh subject, induce voluntary fatigue and record the time. Allow the muscles to rest for five minutes and repeat volitional contractions until fatigue has again occurred. How does the time of fatigue onset, after the rest, compare with that of the first series of contractions?

4. Now, instead of mere rest, give the forearm five minutes' massage and repeat the ergograph experiment. Is the onset of fatigue delayed as compared with the first series of contractions, or with the second, or with both? What is the effect of massage?

XIX. Influence of Tension on the Muscle Contraction. Isometric Contraction.

In the preceding experiments, the resistance offered to the muscle during its contraction, as measured by the weight lifted, has been nearly uniform, of course excepting the inertia of the weight at the beginning of the lift. A muscle twitch under these circumstances

is called *isotonic*. When the shortening of the muscle is prevented by a constantly increasing resistance, so that all its power is used in overcoming the resistance, the contraction is called *isometric*.

The resistance is usually obtained by the use of a spring to which the muscle is attached, the energy of the muscle being stored in the spring in the form of tension, to be liberated as heat as soon as the muscle relaxes.

1. Graduation of the Isometric Spring.—In order to estimate the isometric value of a muscle contracting against resistance offered by a spring, it is necessary to interpret the spring's resistance in terms of weight. Reverse the spring of the heavy myograph (Porter's), attaching its hook to the scale pan beneath. Bring the writing point of the lever against the smoked paper of a drum arranged to be moved by hand. Revolve the drum a half turn to record a base line. Place a 100-gram weight in the scale pan. The spring will be bent to a certain extent and the lever will mark a descending line on the drum. Move the drum, slightly, to record the lower limit of the spring's bend. Repeat with a 200-gram weight, and so on up to 800 grams.

2. Make a gastrocnemius-sciatic preparation. Attach the tendo Achillis to the isometric spring. Adjust the writing point of the lever against the smoked paper of a rapidly revolving drum. Stimulate the nerve with a maximal break shock from an inductorium. An isometric curve will thus be obtained.

3. Release the muscle from the spring and attach it to the ordinary writing lever weighted with 20 grams. The lever, in this case, should be as long as that used for recording the isometric curve. With the drum revolving at the same rate as before, stimulate the nerve so as to record a twitch, as nearly as possible, under the recorded isometric curve.

4. Compare the two curves (1) and (2), as to form and as to work done. To find the amount of tension overcome, as indicated by weight, compare the height of the isometric curve with the depression of the spring in (1).

What influence does tension have on muscle work? How does

this compare with the muscle under isotonic conditions? Is any muscle in the body, normally, under isometric conditions to any extent?

XX. Electric Phenomena of Muscle and Nerve.

1. Galvani's Experiment with Metals.—Pith a frog. Eviscerate and remove everything above the urostyle and the last two vertebræ. Remove the skin from both legs. Pass a hook, made of clean copper wire, under both lumbar plexuses. Suspend the preparation by the copper wire from a clean iron or steel rod. Swing the preparation until some part of it comes in contact with the rod. Result? Explain.

2. Contraction without Metals.—Make a muscle-nerve preparation, cutting the nerve high up. Handle the nerve with a glass hook, allowing the nerve to fall across the muscle. There should be a twitch of the muscle every time the nerve comes in contact with it. This twitch may be due to one of two things. If the muscle is uninjured, and the injured portion of the nerve falls across it, the twitch may be due to the completion of a circuit between the injured and uninjured portion of the nerve itself, which are of different electrical potentials. Or it may be due to the completion of a circuit between injured and uninjured muscle through the nerve. This is known as the *current of injury* or *demarcation current* of muscle or nerve.

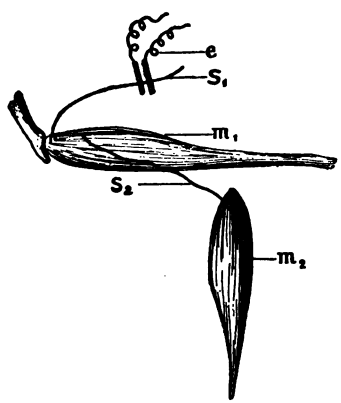

Fig. 14.—Secondary Contraction. s_1, Sciatic of first preparation; s_2, sciatic of second preparation; m_1, muscle of first preparation; m_2, muscle of second preparation; e, electrodes.

3. Secondary Contraction.—Make two muscle-nerve preparations. Allow the nerve of preparation 2 (see Fig. 14) to rest on

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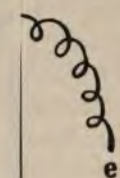

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the muscle of preparation 1. Stimulate the nerve of preparation 2 with a tetanizing current from an inductorium. Both muscles will be thrown into tetanus.

The nerve of the second preparation is stimulated by the *action currents* of the first preparation.

The *action current* is due to a change of potential in an inactive, as compared with an active muscle fibre. The same change may be demonstrated in a nerve over which an impulse is passing. This may be well shown by means of some form of delicate current detector, such as the galvanometer or capillary electrometer.

4. The Capillary Electrometer.—This instrument, as commonly employed, consists of a capillary tube containing mercury and dipping into a vessel containing sulphuric acid. The surface tension of the mercury is so great that it does not flow through the fine capillary tube, and its upper and lower meniscus is convex instead of concave as is the case with water. The sulphuric acid is connected with a platinum wire. The mercury in the capillary is also supplied with a platinum wire for connection with any source of current (see Fig. 15). The upper end of the capillary tube is connected, through a T tube, with a mercury manometer and with a pressure bottle or syringe bulb. By raising the pressure bottle or pressing on the bulb, pressure is exerted upon the mercury in the capillary tube. This pressure is measured by the manometer. By pressing the mercury in the tube downward and then releasing the pressure, some sulphuric acid is drawn up into the tube, into con-

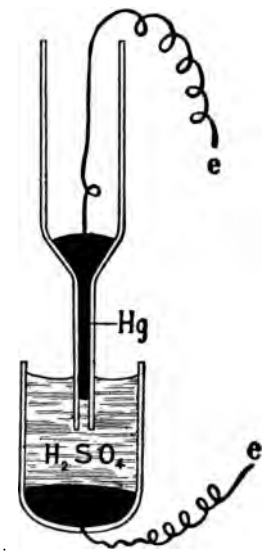

FIG. 15.—Capillary Electrometer. (Lippmann's.) *e e*, wires leading to source of current; *Hg*, mercury in capillary tube; H_2SO_4, sulphuric acid.

tact with the mercury. If a current be passed through the mercury and sulphuric acid, the surface tensions of the fluids are so changed that the mercury meniscus will move in the direction of the current. The extent of the excursion of the mercury is in direct proportion to the strength of the current. This may be measured by mercury pressure as determined by the manometer. A fairly exact graduation of the instrument may be made by placing it in circuit with currents of known strengths, and recording in terms of mercury the amount of pressure needed to bring the meniscus back to its original position. This instrument is exceedingly sensitive to very small currents.

5. Current of Injury of Muscle.—Very carefully dissect out the gracilis and semimembranosus muscles, avoiding crushing or tearing as much as possible. Place the muscle on two non-polarizable electrodes, connected by fine wire with the galvanometer or the capillary electrometer. Test the non-polarizable electrodes first, by bringing them in contact with each other. There should be no deflection of the galvanometer needle or of the mercury meniscus of the capillary electrometer.

(*a*) Interpose a key between the muscle and the current detector. Close the key, so that the galvanometer is brought in circuit with the electrodes on the muscle. There should be but little if any deflection of the needle. If the muscle were absolutely free from injury and at rest, there should be no difference of potential between any of its parts.

(*b*) Cut across one end of the muscle with sharp scissors or scalpel. Place one electrode on the cut surface, the other on the smooth surface. Bring the galvanometer in circuit again. There is now a deflection of the needle. This is an indication of the current of injury or the demarcation current of the muscle.

(*c*) Cut off the nerve near the muscle and repeat (*b*), using the nerve instead of the muscle.

6. Action Current of Muscle.—Make a careful sciatic-gastrocnemius preparation. Place the muscle on the non-polarizable electrodes, connected with the galvanometer. Place the nerve on elec-

trodes from the secondary of an inductorium arranged for single make-and-break stimuli. Close the galvanometer key. There will, probably, be more or less deflection of the needle due to the demarcation current of the muscle which was injured in preparation. With the galvanometer key closed, stimulate the nerve with a single make or break shock. The muscle will respond with a single twitch. Is there any movement of the galvanometer needle? If so, how much and in what direction?

7. Action Current of Frog's Heart.—Pith a frog. Remove the heart, being careful to include the sinus venosus. The heart will, probably, continue its pulsation after its removal from the body of the frog. Place the heart on non-polarizable electrodes, connected with the capillary electrometer. With a low power of the microscope, watch for movements of the meniscus of the mercury in the capillary tube. How many movements can you make out? How do they correspond with the beating of the heart?

8. Paradoxical Contraction.—Pith a frog. Make a sciatic-gastrocnemius preparation, tracing out and cutting the anterior tibial branch of the nerve. Stimulate the cut branch (see Fig. 16). The muscle will contract.

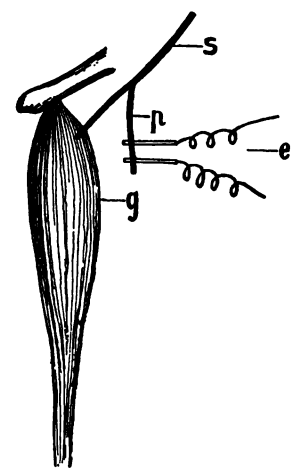

FIG. 16. — Paradoxical Contraction. *s*, Sciatic nerve; *p*, branch to peroneus muscle; *e*, electrodes; *g*, gastrocnemius muscle.

9. Muscle Tone of Rabbit's Gastrocnemius. Demonstration.—Narcotize a fair-sized rabbit with a hypodermic injection of one grain of morphine sulphate. Complete anæsthesia with ether. Tie rabbit, belly down, on the rabbit board, with the hind limbs well stretched out. Make a longitudinal incision through the skin and separate the dorso-lateral thigh muscles. The large shiny white sciatic nerve will be exposed, deep in the wound. Tie a

ligature about the nerve as high up as possible. Cut the nerve above the ligature. Place the cut nerve on shielded electrodes, connected with the secondary coil of an inductorium. Place the primary of the inductorium in circuit with a strong constant current interrupted by the tuning-fork interrupter, vibrating one hundred times per second. Place a short-circuiting key in the secondary circuit. Place the small bell of a stethoscope over the muscle. Open the short-circuiting key. The muscle will be thrown into tetanus and the sound of the vibrating tuning-fork will be heard with the stethoscope. That this reproduction of the tuning-fork tone is really due to the vibration of the muscle fibres to each individual stimulus from the inductorium is shown by the next experiment.

10. Action Currents. Detection of, with the Telephone.— With the same preparation as in the previous experiment, insert needle electrodes into the body and tendinous portion of the gastrocnemius muscle. Connect these with a telephone receiver and again stimulate the sciatic with one hundred shocks per second. You will now hear the sound of the tuning-fork reproduced in the telephone. This is due to the development of action currents in the muscle corresponding, in frequency, to the number of impulses coming to the muscle.

This sound is known as the artificial muscle tone, to distinguish it from the muscle sound which occurs when the muscle is contracted under the influence of volition and which is called the *natural muscle tone.*

This may be heard by placing the stethoscope on the biceps muscle and strongly flexing the forearm on the arm.

XXI. Irritability and Conductivity of Nerve and Muscle During and After the Passage of a Constant Current. Electrotonus.

During the passage of a constant current through a nerve, the irritability and conductivity are increased at the kathode, where the current leaves the nerve, and diminished at the anode, where

the current enters the nerve. Immediately after the cessation of the current, these conditions are reversed; the irritability and conductivity are increased at the anode and decreased at the kathode.

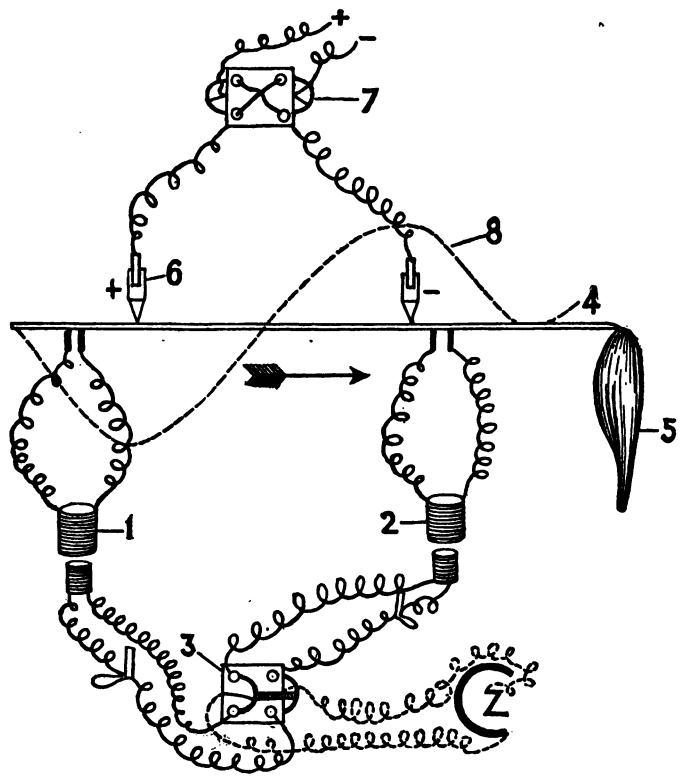

FIG. 17.—Arrangement for Studying Effect of Constant Current on Irritability of Nerve. Current running in direction of arrow is a descending current. Inductoria (1 and 2) connected with battery through current changer (3) in such a way that current may be passed through primaries of either 1 or 2, so as to stimulate nerve (4) in region of anelectrotonus, about anode (6), or in regions of kathelectrotonus, about kathode (8). Nerve impulse indicated by twitch of muscle (5). Through Pohl's commutator (7), with crosspieces in, the constant current may be reversed and become an ascending current, instead of a descending as shown in the figure. The dotted line, running below the nerve at the anode and above at the kathode, represents, respectively, the diminution and increase in irritability of nerve in the anodic and kathodic regions.

This condition of change in a nerve or a muscle, since the muscle itself reacts in the same manner as the nerve, is known as *electrotonus*. The condition of the nerve about the anode is called anelectrotonus; that about the kathode is called katelectrotonus. The conditions of anelectrotonus and katelectrotonus are most marked in the immediate vicinity of the anode and kathode. From these poles they gradually diminish in the extrapolar and interpolar regions, until, in the latter, a neutral point is reached about midway between the two poles (see Fig. 17).

When the kathode is near the muscle and the anode farther from the muscle, the current is said to be descending. When these conditions are reversed, that is, when the anode is near the muscle, the current is said to be ascending. In the following experiments the electrotonic conditions will be tested with the nerve, the muscle twitch being used as a convenient indicator.

1. Make a sciatic-gastrocnemius preparation. Save the whole length of the nerve. Arrange moist chamber with non-polarizable electrodes placed in circuit with one or more battery cells, rheocord, and Pohl's commutator or current-changer. Place the nerve upon the non-polarizable electrodes as shown in Fig. 17. Place a pair of platinum electrodes from the secondary coil of an inductorium on the nerve at the anode of the constant current, and another at the kathode of the constant current. Arrange as in Fig. 17, so that the nerve may be stimulated with the induced current at either pole of the constant current. By means of the Pohl's commutator, the constant current may be reversed in direction; *i.e.*, it may be made either an ascending or a descending current.

Attach the tendon of the muscle to the writing lever of a myograph. Let this record the contractions of the muscle on a drum arranged to be revolved by hand. Send an ascending current through the nerve. While the constant current is passing, stimulate the nerve in the anodic region with a medium strong single-break shock from the inductorium. Mark result of muscular contraction, if any, on drum, as well as data needed to identify what

you have done. Revolve drum a sufficient distance and repeat, stimulating, this time, in the kathodic region. Record data as before.

2. Now, reverse the constant current so that the anode is away from the muscle and the kathode is near the muscle. Repeat the stimulation with the induced current in the anodic and kathodic regions, as before. Record the muscle response, or the lack of it, on the drum and make careful note of all the data.

From these experiments, what conclusions can you draw concerning the effect of the passage of a constant current upon the irritability of a nerve?

3. Repeat the above experiments with ascending and descending currents, stimulating in the anodic and kathodic regions immediately after the cessation of the constant current. What is the after-effect of the passage of the constant current upon the irritability of the nerve?

XXII. The Constant Current as a Stimulus. Pflüger's Laws.

As we have already seen, a sudden increase in intensity of a stimulus which is being applied to a nerve or a muscle is effective in producing an impulse in the same. A gradual increase, on the other hand, is not effective.

In the same way a sudden increase in irritability will, of itself, act as a stimulus. Thus, when a constant current of sufficient strength is passed through a nerve, there is a sudden increase in irritability in the region of the kathode and a sudden decrease in irritability at the anode. There will then be a stimulation of the nerve in the kathodic region which will cause an impulse to be transmitted toward the muscle. If the current is an ascending one, however, and the conductivity is sufficiently diminished at the anode, the impulse will be blocked and the muscle will not respond with a contraction.

When the constant current ceases to flow, the irritability suddenly falls at the kathode and rises at the anode. The conductivity

changes likewise, but not necessarily to the same degree. If the current is ascending and the increase in irritability is sufficient, the muscle will respond with a contraction. If the current is descending, and the fall in conductivity at the kathode is sufficient, this will act as a block between the anode and the muscle and no contraction will be obtained. The relative increase and decrease of irritability and conductivity at the anode and kathode vary with the strength of the contsant current employed. Other things being equal, the make of the constant current is more efficacious as a stimulus than the break.

These facts have been formulated as *Pflüger's laws*. Briefly, they are as follows:

Strength.	Direction of Current.			
	Ascending.		Descending.	
	Make.	Break.	Make.	Break.
Weak	No	No	Yes	No
Medium	Yes	No	Yes	No
Medium strong	Yes	Yes	Yes	Yes
Strong	No	Yes	Yes	No

The words "yes" and "no" in the above table indicate the occurrence or absence of a muscle contraction under the circumstances noted. The strength of the current is regulated by increasing or decreasing the resistance by means of a rheocord or resistance box.

With a fresh muscle-nerve preparation, make and break a constant current through the nerve as indicated by the above table. In the light of the explanations which have been given and the previous experiments performed, how are these results to be explained?

XXIII. Stimulation of Human Nerves.

In human nerves, in the body during life, it is obviously impracticable to bring the electrodes into direct contact with the nerve. There is more or less insulation from the intervening integument

and fat. In this case the electrode, anode or kathode, is brought into as close relation to the nerve as possible. Only a small portion of the current will traverse the nerve, longitudinally. The greater part of the current will traverse the nerve diagonally, forming current loops which spread through the tissues, finally concentrating to pass through the nerve again to the other electrode.

Those points at which the current enters the nerve are known as physiological anodes, and those where the current leaves the nerve, as physiological kathodes. Thus, at each pole groups of physiological anodes and kathodes are found. The contraction of the muscle which occurs when the current is closed represents irritation at the physiological kathode. That contraction occurring at the break of the current represents irritation at the physiological anode.

Since there are both physiological anodes and kathodes at each pole, any one or more of the following results may be obtained through the opening or closing of the constant current:

1. **Anodic Closing Contraction.**—Contraction following the change developed at the physiological kathode beneath the physical anode.

2. **Anodic Opening Contraction.**—Contraction following the change produced in the nerve at the physiological anode beneath the physical kathode.

3. **Kathodic Closing Contraction.**—Contraction following the change produced in the nerve at the physiological kathode beneath the physical kathode.

4. **Kathodic Opening Contraction.**—Contraction following the change produced in the nerve at the physiological anode beneath the physical kathode.

The following abbreviations for these contractions, from 1 to 4 respectively, are used: ACC, AOC, KCC, KOC.

KCC and ACC are stronger than KOC and AOC. KCC is stronger than ACC, and AOC is stronger than KOC.

The effect of change of strength of current is shown in the following table:

Weak Currents.	Medium Currents.	Strong Currents.
KCC	KCC	KCC
———	ACC	ACC
———	AOC	AOC
———	———	KOC

With a strong current, tetanus sometimes occurs both at the make and the break.

1. Experiment.—Set up 8 or 10 dry cells in series. Place a commutator and simple key in circuit with the brass electrodes to be used for the stimulation of the human nerve. Place the anode electrode on the back of the neck and the kathode over the ulnar nerve at the elbow.

(*a*) Make and break the circuit with the simple key. If there is no accompanying muscular contraction, add more cells to the circuit until a contraction is obtained. Record results and compare with the table given above.

(*b*) Change the direction of the current by means of the commutator key so as to make the electrode, over the nerve, the anode.

Again make and break the circuit, starting with weak currents and increasing the strength of current as before. Keep a careful record of the various strengths of current and the appearance of the different contraction responses.

2. Reaction of Degeneration.—In a muscle whose nerve has been cut off from its controlling cell, after a time certain definite changes in irritability to the constant and induced currents occur. There is a gradual diminution in excitability to the induced current and at first an increased excitability to the constant current. Later, this diminishes also. The muscle contraction may also become greatly prolonged and a condition called galvanotonus (tonic contraction) may be easily produced. The normal contraction formula is departed from, the most characteristic change being a reversal of the usual appearance of KCC and ACC. Normally,

KCC appears before ACC. In well-marked degeneration ACC appears first.

Experiment.—Narcotize a rabbit with morphine and ether. Expose and cut one sciatic nerve as high up as possible. Sew up the wound and test the muscle at intervals of two days for the reaction of degeneration.

XXIV. Action of Certain Drugs upon the Single Muscle Twitch.

1. Veratrine.—Make a saturated solution of veratrine in 0.6-per-cent sodium-chloride solution. Pith a frog and inject, under the skin, about five drops of the veratrine solution. In a few minutes (ten to fifteen), if the frog be made to jump, it will be seen that the recovery of the flexed position of the hind limbs is very slowly brought about. A little later, a spasm of both limbs occurs at every attempt to jump. The flexor muscles, being the weaker, are overpowered by the extensors.

Make a gastrocnemius-sciatic preparation from the frog. Attach the tendon to the writing-lever of a myograph. Adjust the point of the lever to the smoked paper of a drum revolving at medium speed. Stimulate the nerve with a single maximal break shock from an inductorium. How does the recorded contraction compare with the normal muscle twitch?

2. Adrenalin (active principle of the suprarenal gland).—Inject into the dorsal lymph sac of a pithed frog 10 drops of a 1 to 10,000 solution of adrenalin chloride. After ten minutes, make a muscle preparation and proceed as in 1. Is there any departure from the normal muscle twitch? Prepare the muscle and nerve of the other leg or of another frog. Immerse the muscle in the adrenalin for a few minutes and again record a contraction.

XXV. Involuntary Muscle.

Synonymes: *Plain* or *smooth*, *slow*, *non-striated*. 1. Remove the stomach from a pithed frog. Make two parallel cuts through the viscus running at right angles to the long axis and about one-half

centimetre apart. Pass a bent pin through the ring thus made, and support this in the femur clamp of the myograph. Pass a fine copper wire through the lower portion of the muscle ring and attach it to the muscle lever and the binding post of the lever. Connect this post and that of the femur clamp with a dry cell, interposing a simple key and a signal magnet in the circuit. Adjust the point of the muscle lever, the point of the signal magnet, and the point of a chronograph lever, marking hundredths of a second, in a straight perpendicular line against the smoked paper of a drum arranged to revolve at high speed. Start the drum, place the chronograph in circuit with the vibrating tuning-fork, open and close the key in the battery circuit.

Compare the length of the latent period and the form of the contraction curve with that of skeletal muscle.

2. Make a second similar preparation, omitting the electrical apparatus for stimulation, and allow the lever of the myograph to rest against a drum revolving once an hour.

Observe tonic contractions of the muscle.

CHAPTER III.

NERVOUS SYSTEM.

I. Reflex Action.

Chloroform a frog. Make a longitudinal incision through the skin in the middle line of the skull. Cross this with another incision from ear drum to ear drum. Turn back the skin flaps and expose the skull. Carefully remove this piecemeal with strong scissors and forceps from before backwards. Expose the brain, noting its relations to the landmarks on the skull. Compare with Fig. 18.

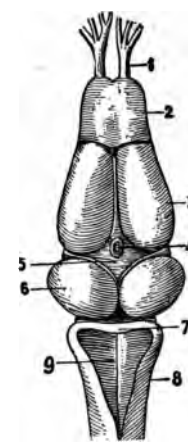

Fig. 18.—Frog's Brain. 1, Olfactory nerves; 2, olfactory lobes; 3, cerebral lobes; 4, epiphysis cerebri (pineal body); 5, optic thalamus; 6, optic lobes; 7, cerebellum; 8, medulla; 9, rhomboid fossa (fourth ventricle).

1. Reflex Action with Cerebrum only Removed.—Partly anæsthetize a frog. Cut through the skull with sharp scissors or scalpel, transversely, just in front of the ear drums. This will serve to eliminate the cerebral lobes. Clamp the lower jaw in a femur clamp and suspend the frog from an upright stand. Keep the wound made, moist with physiological salt solution. Allow the frog time to recover from the shock of the operation and try the following experiments:

(*a*) Immerse one foot in a beaker containing a dilute solution of sulphuric acid (1 to 10,000). Note the time elapsing between the application of the stimulus and the first muscular contraction. Which muscles contract first? Does the reaction extend to any

other muscles if the application of the stimulus is continued? Explain.

(*b*) Wash the foot thoroughly in plain tap water and dry with filter paper. Repeat experiment (*a*), using a stronger solution of the acid (1 to 1000). Record reflex time as before.

(*c*) Wash the feet again and dry with filter paper. Repeat observations, using a still stronger solution of acid (1 to 500), and record results. What is the effect on reflex time of increasing the strength of the stimulus?

(*d*) Instead of the acid, use medium make-and-break shocks from an inductorium as the stimulating agent. Make and break every three seconds for ten shocks. Is there any reflex response?

(*e*) Increase the frequency of stimulation to one per second; to two per second. Is there any reflex? If so, how many stimuli must be applied to the skin before the reflex arc is completed? Where are the main places of resistance in the reflex arc?

2. Reflex Action with Optic Lobes also Removed.—Using the same frog as in the previous experiments, make an incision through the skull and brain just behind the tympanic membrane. Allow time for the nervous system to recover from the shock of the operation before proceeding with the next series of observations.

Repeat experiments (*a*) to (*e*) of series 1. How does the reflex time of this set of experiments compare with that of the previous set?

What is your conclusion concerning the influence of the optic lobes, in the frog, on cord reflexes?

3. Reflex Action with Medulla Removed.—Complete the pithing of the frog and repeat the previous series of experiments. Conclusions?

4. Diffusion of Impulses within the Cord.—Pith another frog. Suspend as before. Apply a strong and continuous stimulus to one foot. Note the successive groups of muscles that become involved in the reflex reaction. Also note the time at which each group becomes involved and the order of response.

5. Apparent Purposive Character of Reflex Responses.—A pithed frog is prepared as in the previous experiments. Take a small piece of filter paper wet with acetic acid diluted one-half with water, and apply this to the ventral aspect of one thigh. Note the attempt to remove this with the foot of the same side. If this is unsuccessful, or if the leg be held fast, the foot of the opposite side will be brought into play and even the fore limbs, in an attempt to brush off the offending irritant.

6. To Show the Centres of Reflex Exchange in the Decerebrized Frog.—Using the frog of the previous experiment, run a long needle through the neural canal to destroy the spinal cord. After a sufficient interval, test the frog as before for reflexes. Result? What is the function of the cord in relation to reflex action?

Test the excitability of the muscles and nerves with the induced current. Open the thorax and observe the beating of the heart. The frog is still alive so far as the vegetative functions are concerned, but the entire cerebro-spinal axis is destroyed and consequently all reflex action is abolished.

7. Action of Strychnine.—Decerebrize a frog. Test the reflexes as in experiment 2. Now inject under the skin a solution containing one-half milligram of strychnine sulphate. Test reflexes again, as before, at five-minute intervals. If there is no appreciable change after ten minutes, repeat the injection. What is the effect upon reflex time as compared with the normal for the frog used?

Repeat the injection until the frog is thrown into tetanic spasms upon the slightest stimulation. What is the character of these spasms? What is the position of the frog during a convulsion? Explain.

Now destroy the cord by passing a needle through the neural canal. What is the effect upon the strychnine spasms? What is the seat of the strychnine action?

8. Action of Chloral Hydrate.—Pith another frog. Establish the normal reflex time to some stimulus, taken as a standard. Inject under the skin 10 drops (about 0.6 c.c.) of a 2-per-

cent solution of chloral hydrate. Test the reflexes as before and compare with the normal taken as a standard. How does the effect of the chloral compare with that of the strychnine? Repeat the injection of the chloral until a lethal effect is produced. Note all accompanying phenomena.

II. Reaction Time.

1. For Sound.—A tuning fork vibrating one hundred times per second is placed in circuit with a time-marker. Two short-circuiting keys are placed in circuit with the time-marker, so that, by closing either one, the time-marker may be cut out of the circuit. The student experimented upon holds the handle of one key. Another student holds the handle of the other key. The first key is held open, the second key being closed. Let the subject of the experiment close his eyes. He should close his key as soon as he hears the opening click of the other key.

The opening of the one key sets the time-marker to recording. The closure of the other key stops the time record. This should be taken upon a rapidly revolving drum. The interval between the opening of the circuit and its closure is marked in hundredths of a second and represents the time occupied for the passage of the sound wave through the auditory apparatus, the auditory nerve to the auditory centres of consciousness; its transfer to a motor neuron; its passage to the muscles involved and the latent period of these muscles.

Repeat the experiment ten times for each individual and try a number of different individuals. Estimate the average reaction time for each individual.

2. For Vision.—For the first key to make the circuit, use a mercury contact. Darken the room, so that the spark made when the key is opened may be distinctly seen. Let the subject of the experiment close his key as soon as he sees the spark made by the opening of the other. The interval marked by the tuning fork gives the reaction time for vision. This experiment should also be repeated ten times and the average taken.

3. For Tactile Sensation.—The circuit-opening key may be so arranged that, upon opening, it will come into sharp contact with the finger of the subject who is to close his key as soon as he feels this contact. This will give the reaction time for tactile sensation.

4. Influence of Drugs on the Reaction Time.—Repeat experiments 1 to 3 on one of the subjects whose normal reaction time has already been determined, after drinking 30 c.c. of whiskey diluted with equal parts of water.

III. Removal of Cerebrum in the Frog.

Remove the cerebral hemispheres in a frog by making a cut through the skull in front of the tympanic membranes. Compare

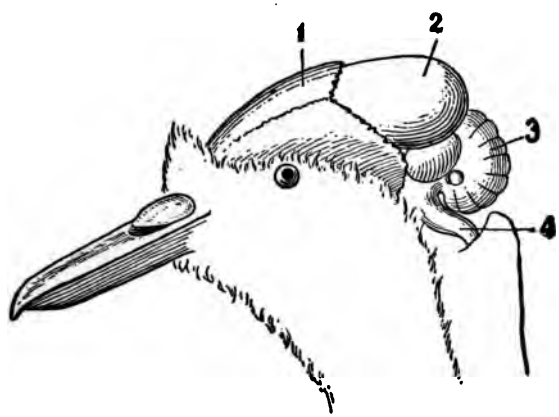

Fig. 19.—Pigeon's Brain. 1, Skull which has been removed in part to show relations of cerebrum (2), cerebellum (3), and medulla (4).

the frog so treated with a normal frog. Place both the normal and the decerebrized frogs on their backs. How do they react? Stimulate the decerebrized frog. Is there any change in the co-ordination of movements? Place both frogs in water. Can the decerebrized frog swim in a normal manner?

Make cut behind the tympanic membranes. Compare this condition with the preceding and with the normal.

IV. Removal of the Cerebrum in a Pigeon.

Remove the cerebral hemispheres in a pigeon anæsthetized with ether. Sew skin over wound. Allow the pigeon twenty-four hours to recover from the effect of the operation. Compare the pigeon so treated with a normal bird. Is there any disturbance of co-ordination? Is the pigeon able to sit on a perch? Is it able to fly?

V. Removal of the Cerebellum of a Pigeon.

Etherize another pigeon. Remove the bone of the skull over the cerebellar region, leaving a bony bridge in the middle line. Go in from either side with a blunt instrument. Sew skin over wound and allow twenty-four hours for recovery from the shock of the operation.

How does this pigeon compare with the normal and with the one which has had its cerebrum removed? Describe all phenomena and reactions to various stimuli. Are there any disturbances of co-ordination? If so, what are they?

VI. Hemisection of the Spinal Cord.

Narcotize a dog or a rabbit with morphine and ether. Cut off hair of back in upper lumbar region. Make a longitudinal incision through the skin and muscles over the spinous processes of the first three vertebræ. Cut through the spinous processes with bone-scissors. Clean the laminæ of muscle. With a small trephine, carefully remove a button of bone from the lamina of one side. From this opening remove the rest of the lamina with fine bone-cutting forceps. This will expose the cord in its membranes. Make an incision through the membranes with fine-pointed scissors. With a fine sharp scalpel cut through one-half of the cord. After any ensuing hemorrhage has been controlled, sew up the wound with catgut or silk. Allow the animal twenty-four or thirty-six hours to recover from the shock of the operation and then make observations on the affected side compared with the other for changes in voluntary motion and sensation. Test the various reflexes also

and compare with the normal. Observe the animal from day to day and note any change in motion or sensation. Try the muscles for the reaction of degeneration.

VII. Stimulation of the Motor Areas of the Dog's Brain. Demonstration.

Lightly narcotize a dog with morphine and anæsthetize with ether. Tie on dog-board, belly down, with head well stretched out and supported on a block of wood placed beneath it. With the trephine remove a button of bone from one parietal. This opening may be enlarged by repeating the trephining several times. The remainder of the bone may then be removed piecemeal with bone forceps and scissors. Expose in this manner the whole lateral and dorsal aspect of one cerebral hemisphere. Identify the fissures and motor points as shown in Fig. 20.

With fine platinum electrodes, having the two poles but slightly separated, stimulate at the points indicated in the figure. The current used for this purpose is a tetanizing current from an inductorium of medium strength.

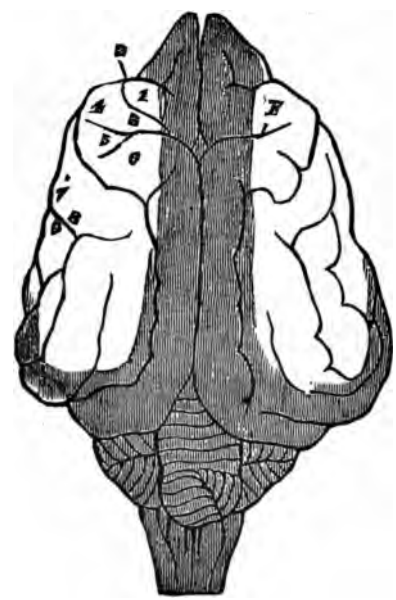

Fig. 20. — Dog's Brain, Showing Various Motor Areas. *F*, Frontal fissure, sometimes termed crucial sulcus, corresponding to the fissure of Rolando in man. 1, Flexion of head on neck in median line; 2, flexion of head on neck with rotation towards side of stimulus; 3, 4, flexion and extension of anterior limb; 5, 6, flexion and extension of posterior limb; 7, 8, 9, contraction of orbicularis oculi, and facial muscles in general. The unshaded part is that exposed by opening the skull. (Dalton.)

VIII. Division of the Semicircular Canals.

A young pigeon serves best for this purpose. It is well to make a dissection on a dead bird first in order to become familiar with the position and relations of the canals. Make a transverse incision through the skin of the head. Slip these flaps back so as to expose the bone. Scrape away the insertions of the neck muscles.

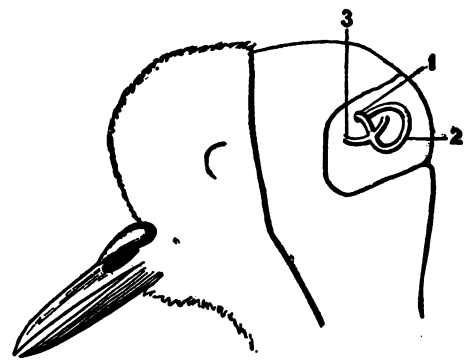

Fig. 21.—Semicircular Canals (Pigeon). Outer plate of skull and cancellous bone removed to expose the semicircular canals: 1, Superior (vertical); 2, posterior (vertical); and 3, anterior (horizontal). The planes of 1, 2, and 3 cut each other at right angles.

Remove the outer table of the skull behind each ear, carefully removing with the forceps the cancellous or spongy bone between the two plates until the canals are seen (see Fig. 21).

Having made the preliminary dissection on a dead bird, repeat the same process on a live pigeon under the influence of chloroform. After the canals are exposed, cut through one or two of them, with strong scissors, making a careful record of the canals thus injured. Control the bleeding with suprarenal extract.

If the bird recovers from the immediate effects of the operation, carefully observe and note its departure from the normal condition of the pigeon with the semicircular canals intact. Compare the behavior of this pigeon with that of the pigeon which had its cerebellum removed.

IX. To Determine the Number of Impulses Discharged by a Nerve Cell in a Given Unit of Time.

In an etherized rabbit, adopting the same methods as were employed in the experiment on hemisection of the cord, expose the cord in the middle lumbar region. Insert needle electrodes in the gastrocnemius muscle of one side. Connect these with the capillary electrometer. Insert fine needle electrodes through the cord. Stimulate the cord with medium strong single induction shocks at intervals of one second. Is there any response of the gastrocnemius muscle? If so, what is the nature of this response? Increase the frequency of stimulation of the cord and note results. Stimulate the cord ten times per second. Does the muscle go into tetanus? Does the muscular contraction continue after the stimulation of the cord has been stopped? What changes occur in the capillary electrometer?

Place the tuning-fork interrupter in circuit with the primary coil of the inductorium. Stimulate the cord again. Does the muscle go into tetanus? What is the frequency of vibration of the meniscus of the capillary electrometer?

Listen with a stethoscope to the muscle during contraction. Is the tone of the tuning-fork reproduced in the muscle? If not, is there any sound heard in connection with the contraction?

Compare these results with those obtained when the sciatic nerve was stimulated in the experiment on muscle tone. What is the rate of discharge from the nerve cells in the cord?

CHAPTER IV.

BLOOD.

THE blood may be looked upon as the common carrier of the body. It serves to carry food stuffs to the tissues from the alimentary canal where they have been absorbed and O and CO_2 between the lungs and the tissues. It also carries away from the tissues waste products, resulting from their metabolism, to the organs of excretion. It acts as a medium of exchange between the tissues themselves, carrying products of glandular activity from one group of cells to another, as in the internal secretions. It is a prime factor in the regulation of body temperature. It is finally, in part, the receptacle for and, in part, the seat of the formation of protective substances which are manufactured by the body as a result of the introduction of toxins from without.

In structure, the blood consists of two main elements, a liquid portion or *plasma*, and a cellular portion, *corpuscles*. The latter are divisible into two classes, the colored corpuscles or erythrocytes and the colorless corpuscles or leucocytes. They are also known respectively as the red and white corpuscles. Their numbers, varieties, and properties will be considered later.

I. COAGULATION OF THE BLOOD.

Narcotize and etherize a dog or rabbit. The former will furnish more blood. Expose both carotid arteries. Introduce a cannula into each carotid, securing the arteries on the side near the heart with artery clamps.

1. Prepare a series of test tubes, as follows: (*a*) Clean empty tube for receiving a sample of fresh shed blood; (*b*) tube half full

of distilled water; (*c*) tube half full of 0.8-per-cent NaCl solution; (*d*) tube half full of saturated NaCl solution; (*e*) larger tube quarter filled with a saturated solution of $MgSO_4$.

Open clamp on one carotid and complete the filling of the test tubes with blood. Set tube (*e*) to one side for use later. Observe what happens in tube (*a*), which contains undiluted fresh blood. How long before the blood in tube (*a*) is completely solidified? Invert test tube. The blood does not run out, but adheres to the sides of the tube as a jelly-like mass of the same volume and color throughout as when first shed. Later, the mass shrinks, the surface becoming cup-shaped and, as the shrinking continues, more and more of a clear straw-colored fluid collects upon it. This is the *serum* which is not subject to further coagulation, except that caused by high temperatures in any albuminous fluid. The solid mass remaining finally floats in the serum as this accumulates; it consists of a stringy substance, *fibrin*, and blood corpuscles entangled in its network-like meshes.

If a little fresh blood be allowed to drop on a glass slide and is then covered with a cover slip and placed in a moist chamber to prevent drying, after fifteen to twenty minutes the fibrin fibrils may be seen with a low power of the microscope.

Has the distilled water of tube (*b*) any effect in hastening or delaying the coagulation of the blood shed into it?

Has the 0.8-per-cent NaCl solution of tube (*c*) any effect in hastening or delaying the coagulation?

What is the effect of the saturated salt solution of tube (*d*)?

2. Compare the color of the fresh undiluted blood with the saturated salt-solution dilution and the distilled-water dilution. Compare the different tubes in transmitted and reflected light. To what is the opacity of the fresh blood due? To what is the transparency of the water-diluted blood due?

3. Place a specimen from each tube under the microscope and compare the appearances of the red corpuscles. With distilled water and some other reagents the red blood corpuscles lose their pigment (hæmoglobin) which goes into solution in the diluted

plasma, giving it a transparent color. This process is known as *laking* and the blood is said to be laked. The corpuscles will appear faintly outlined as ghost or shadow corpuscles.

4. The magnesium sulphate of tube (*e*) prevents coagulation. Let the mixture stand in a cool place until the corpuscles have settled to the bottom of the tube. The supernatant liquid, the "salted plasma," may then be pipetted or siphoned off.

Divide this salted plasma into four portions. To each portion add eight times its volume of water. To portion 1 add a few drops of a half-per-cent solution of ammonium oxalate. To portion 2 add a little of the clot from tube (*a*). Portion 3, place in a water bath heated to 38° C. Place portion 4 on a water bath heated to 60° C. and add a few drops of ammonium oxalate.

Observe the presence or absence of the phenomena of coagulation in the portions of salted plasma treated as above. Are calcium salts necessary to coagulation? What is the effect of temperature on coagulation? Why does coagulation take place in portion 2?

To tubes 1 and 4 add a few drops of calcium chlorid. What is the effect as far as coagulation is concerned?

There are various theories to explain the coagulation of the blood. The known facts are as follows: Clotting is produced through the formation of a coagulated substance, *fibrin;* for the formation of fibrin three things are necessary: a globulin, *fibrinogen*, *calcium salts*, and an enzyme, *fibrin ferment* or *thrombin*.

Fibrinogen and soluble calcium salts are normally present in the blood plasma. Thrombin is formed at the time of coagulation. The mooted question is the origin of the thrombin. The thrombin is a nucleo-proteid which seems to be formed through cell disintegration and especially through the breaking down of leucocytes.

5. Defibrination of Blood.—To defibrinate blood, collect it from a bleeding artery, in a shallow vessel. As the blood is shed, whip or beat it, vigorously, with a glass rod or a bundle of twigs. The fibrin, as it is formed, separates from the blood and adheres to the whip as a sticky, stringy, almost colorless mass. The blood

so treated is then filtered through a fine-mesh cloth. Defibrinated blood will not clot spontaneously.

II. The Number of Red and White Blood Corpuscles.

1. Counting the Erythrocytes or Red Corpuscles.—The Thoma-Zeiss hæmocytometer is used for this purpose. This con-

FIG. 22.—Thoma-Zeiss Hæmocytometer Counting-chamber. *s*, Glass slide, upon which is mounted a covered disc *m*, accurately ruled to present one square millimetre divided into 400 squares. This is surrounded by another annular cell, *c*, which projects in height exactly one tenth of a millimetre above *m*.

sists of a graduated pipette for accurately diluting a known quantity of blood with some fluid having the same osmotic pressure as the blood. One of the most satisfactory diluents is physiological salt solution. For human blood, this consists of a solution of sodium chlorid, 8.5 grams in 1000 c.c. of water.

The capillary stem of the pipette, used for diluting the blood for counting the red corpuscles, has a capacity equalling one-hundredth of the hollow ball with which it joins (see Fig. 23). If the blood is drawn up to the line marked 1 on the pipette stem and then the diluent drawn in until the mixture reaches the line 101

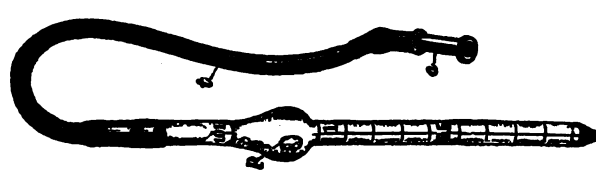

FIG. 23.—Thoma-Zeiss Hæmocytometer Pipette.

marked just above the ball (see Fig. 23), there will be 101 parts of fluid, of which the blood forms 1. The contents of the stem, however, do not have to be considered after the dilution is made, since they can be displaced, unmixed. The dilution of the blood will then be 1 to 100. As the blood and diluting fluid enter the mixing

chamber, this should be constantly rotated between the fingers so as to facilitate the mixture and avoid error through coagulation.

The other part of the instrument is the micrometer slide upon which the diluted blood is evenly spread for counting the corpuscles. This consists of a glass slide (Fig. 22), upon which is mounted a covered disc, *m*, a square millimetre of which is subdivided by a dividing engine into 400 squares of one-twentieth millimetre each. The micrometer, *m*, is surrounded by an annular cell, *c*, the sides of which project one-tenth millimetre above the surface of *m*. This cell is closed by a thin flat glass cover, so that the cubic space included between each small square of the micrometer and the cover would be $\frac{1}{4000}$ of a cubic millimetre.

To find the number of corpuscles in a cubic millimetre of undiluted blood, multiply 4000 by the dilution and this by the total number of corpuscles counted. This result is then divided by the number of small squares counted. If the blood has been drawn only to the 0.5 mark in the diluting pipette, the blood dilution is 1 to 200 and this number must be substituted for the factor 100 in the formula given above. With normal blood, the higher dilution is advisable.

Procedure.—Thoroughly cleanse the tip of the finger or, preferably, the lobe of the ear, with soap and water. Wipe off with a cloth wet with alcohol. Dry thoroughly. With a sterilized needle, or a sharp pen with one nib broken off, make a quick stab of the ear or the finger. Wipe off the first drop of blood. Blood should ooze freely from the puncture without pressure. Insert the point of the pipette well into the blood drop and carefully draw in blood to the 0.5 mark on the stem of the pipette. With a cotton cloth wipe off all blood adhering to the outside of the pipette. Dip the end of the pipette into the diluting fluid and draw this in through the stem and into the ball until the 101 mark is reached. The pipette should be gently rotated while the filling is going on, in order that the mixture of the blood and diluting fluid may be assured through the movements of the glass bead in the ball. Close both ends of the pipette with thumb and forefinger and shake well.

This is to obtain a uniform distribution of the corpuscles throughout the mixture.

To Fill the Counting Cell.—Blow out three or four drops of the diluted blood from the pipette. Now allow a small drop to flow upon the disc of the counting chamber. Cover quickly, pressing the cover gently down until Newton's rings are seen. These are the spectrum colors due to refraction between the two layers of glass. They do not appear if there is any fluid or dirt between the cover and the cell. If any fluid runs over into the moat between the cell and the micrometer, the slide will have to be cleaned and another drop of the diluted blood taken. Repeat until a satisfactory specimen for counting is obtained.

Allow several minutes for the corpuscles to sink to the bottom of the cell upon the ruled squares. It is obvious that the counting cell must ke kept in the horizontal position. Place this upon the stage of a microscope and count the corpuscles in all the squares. For convenience of counting, the micrometer is divided into sixteen large squares by double lines, and these, in their turn, are subdivided into the small squares already mentioned. Count several specimens, in this way, and take the average. Compare the blood of various students.

To Clean the Cell and Pipette.—The cell should be carefully rinsed with distilled water and dried with a soft cloth or absorbent cotton. The cover should be treated in the same way. Neither alcohol nor ether should be used since they will coagulate the albumin of the blood. In cleaning the pipette first blow out any blood mixture remaining. Fill with distilled water several times. If all traces of blood are not removed in this way, rinse with an aqueous solution of hydrogen peroxide and again wash out with distilled water. Now draw alcohol through by suction and follow this with ether, drawing through a stream of air until the pipette is thoroughly dry. This is manifest when the glass bead enclosed in the bulb of the pipette no longer adheres to the sides.

Counting the White Corpuscles.—Since there is a much smaller number of leucocytes than of red corpuscles, the dilution required

is much less. A special pipette is employed, with which a dilution of 1 to 10 or 1 to 20 may be obtained. The diluting fluid employed is usually a 0.2 of one per cent acetic acid. This accentuates the

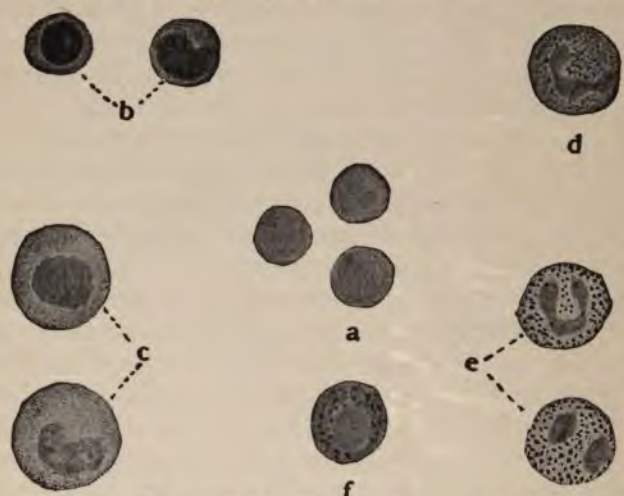

FIG. 24.—Human Blood-corpuscles. *a*, Red blood-corpuscles for comparison; *b*, small hyaline cell or small lymphocyte; *c*, large hyaline cell or large lymphocyte; *d*, fine granular oxyphile; *e*, coarse granular oxyphile or eosinophile; *f*, basophile. (F. C. Busch.)

nuclei of the white cells and decolorizes the reds. Aside from this, the technique of counting is the same as that for the reds.

II. CHANGES PRODUCED IN THE CORPUSCLES THROUGH VARIATIONS OF OSMOTIC PRESSURE.

Dilute equal volumes of defibrinated blood with (*a*) distilled water; (*b*) 0.8-per-cent sodium-chlorid solution; (*c*) 5-per-cent sodium-chlorid solution.

Note the color and opacity of (*b*) and (*c*) as compared with (*a*). Place samples of the three preparations under the microscope. Note the changes of form and color of the corpuscles: (*b*) is an isotonic solution for the blood, (*c*) is hyperisotonic, and (*a*) is hypoisotonic.

With fresh preparations on a slide and covered with a cover glass, try the effects of the following reagents: acetic acid, chloroform, ether.

Place another specimen of defibrinated blood in a freezing mixture.

III. Microscopic Examination of the Blood.

Appliances.—Microscope, with a substage condenser, a high dry and an oil-immersion lens. A stage micrometer. An eyepiece micrometer. Slides and cover slips. Staining reagents, consisting of methylene blue (aqueous solution), eosin (sat. aqueous solution), Wright's stain.

The slides and cover slips used must be scrupulously clean. With new slides, washing with soap and warm water, and then rinsing in distilled water, are generally sufficient. The glasses should then be wiped dry with a clean soft linen or cotton cloth. Handling the surfaces of the slides or cover slips with the fingers should be avoided, since the oil from the skin deposited in this way is hard to remove and prevents an even spreading of the blood-film. Before using the slides or cover slips, they should be gently warmed for a moment in the flame of the alcohol lamp or Bunsen burner in order to get rid of any water of condensation.

1. In the manner described before, obtain a drop of blood from the finger or ear. Touch the middle of a clean glass slide to the drop so that a small portion adheres to the slide. Avoid touching the skin of the ear with the slide. Gently place a clean cover slip over the drop and allow the blood to spread out in a thin film between the slide and the cover glass without using any additional pressure.

Examine this fresh specimen under the microscope with the high dry lens. Note the color and form of the red cells. Compare them with the leucocytes in relative number and form. Are the red cells nucleated? What is the appearance of the red cells in profile? Do the red cells vary in size and form? If so, to what may these variations be attributed?

2. Instead of the blood slide, place the stage micrometer under the microscope. With the help of this, find the exact value of the spaces between the lines of the ocular micrometer. Remove the stage micrometer and again place the blood specimen under the microscope. With the ocular micrometer, measure the dimensions of twenty-five red corpuscles. Compare the size of the white corpuscles with that of the reds.

3. Pith a frog. From the wound thus made, secure some blood for microscopical examination. How do the red blood cells of the frog compare in size, form, and nucleation with the red cells of man and of the rabbit or dog? In frog's blood what is the relative size of red and white cell?

IV. Staining of the Blood Cells and Differential Count of the Leucocytes.

A dried blood smear or film is usually employed for this purpose. The blood smear may be made in one of two ways, either on a slide or upon a cover slip. If the former method is employed, the smear is allowed to dry on the slide, is stained, and examined, without the use of a cover glass. If the latter method is used, the smear is dried, fixed, and stained upon the cover slip and this is then inverted, smear side down, upon a slide over a drop of balsam or a shallow air cell.

1. The Smear on the Slide Direct.—Take two slides. Touch the edge of one to the drop of blood. With this slide forming an angle of about 25° with the other, quickly and firmly apply its blood-stained edge to the other slide and sweep it over the surface.

2. The Cover-Glass Smear.—For this purpose, two cover slips are used. One of the cover slips is carefully applied to the drop of blood, so that a drop adheres to the centre of the slip. This slip is then applied to the other and the blood allowed to spread in a thin film between the two. After this has occurred, the two slips are carefully drawn apart and the smears allowed to dry in the air.

3. Staining the Films.—The simplest and best available blood-staining reagent at the present time is that devised by

Wright. This is a modification of the method used by Jenner. The method is based upon the fixing and solvent powers of methylic alcohol. The essential pigments of the stain are polychrome methylene blue and Grübler's yellow eosin.

(*a*) Cover the blood film on the slide or cover glass with as much of the stain as it will hold. Allow this to remain undisturbed for about one minute. By this time the film is fixed upon the slide.

(*b*) Now add water, drop by drop, until the surface of the stain assumes a greenish metallic tinge. Allow the stain to remain on the cover slip for two minutes longer.

(*c*) Wash in tap water for two minutes or until the smear has acquired a yellowish-pink hue. The water serves to differentiate the stain and wash out the excess of blue.

(*d*) Dry the specimen carefully with filter paper and mount in Canada balsam.

4. Iodine Reaction.—The reaction which iodine gives with the finely granular oxyphile cells or polynuclear neutrophiles, is known as *iodophilia*. The following solution is employed:

Iodine	1 gm.
Potassium iodide	3 "
Water	100 c.c.
Gum Arabic	50 gm.

Place a drop of this mixture upon a slide. Take a fresh blood film on a cover slip and press it, film down, upon this mixture. Squeeze out from under the cover slip the excess of the mixture, so that the remaining film is sufficiently thin to avoid obscuring the corpuscles through too deep a color of the mounting medium.

In normal blood, all the cells will be tinged a bright yellow: the reds, uniformly; the whites, with a more refractile nucleus. In certain pathological conditions, with this treatment of the blood, reddish-brown granules appear in the cytoplasm of the polynuclear neutrophiles as well as granular masses of a similar tinge outside of the cells.

V. Classification of the Leucocytes.

There is, at present, no perfectly satisfactory classification of the white cells. One of the most logical is that of Kanthack and Hardy (see Fig. 24). This is a classification according to reaction to staining reagents and to presence or absence of granulation and is as follows:

A. Oxyphile (staining with acid dyes).
   1. Finely granular.
   2. Coarsely granular.
B. Basophile (staining with basic dyes).
   1. Finely granular.
C. Hyaline.
   1. Small.
   2. Large.

The more usual classification, however, is as follows: (*a*) polymorphonuclear neutrophiles, (*b*) eosinophiles, (*c*) mast cells, (*d*) large mononuclear cells, (*e*) lymphocytes (large and small).

Of these, (*a*) to (*d*) inclusive originate in the bone marrow. Group (*e*) comes from adenoid tissue.

1. Differential Count.—Study one of the best stained specimens of human blood until you are familiar with the different forms of leucocytes enumerated above. Now go over the specimen carefully and systematically, using a mechanical stage so as not to go over the same field twice, and keep count of the number of individuals of the different varieties. Count five hundred cells in all and estimate the percentage of each form. At the same time keep careful watch for any abnormalities of the reds.

Stain a specimen of frog's blood and compare the varieties of white cells with those found in human blood.

Stain smears of human blood with eosin and methylene blue, after fixation for two hours in a mixture of alcohol and ether, equal parts.

Normal Percentage of Each Variety (Cabot).

Small lymphocytes	20–30	per cent.
Large "	4–8	"
Polymorphonuclear neutrophiles	62–70	"
Eosinophiles	½–4	"
"Mast cells"	1/10–9/10	"

Description of the Different Varieties.—The variety which is most numerous, as may be seen from the above table, is the so-called polynuclear or polymorphonuclear neutrophile. With Wright's stain, the nucleus of this corpuscle takes an intense navy-blue color and is sharply defined. The nucleus is irregular in outline and may assume a great variety of forms. There may be two or more nuclear masses united by finer bands of nuclear substance. The cytoplasm contains fine granulations which take, with Wright's stain, several different shades of pink. Where the simple eosin and methylene-blue staining is employed, these granules take a faint pink tinge.

The lymphocytes, which, in point of number, come next to the polynuclear neutrophile, vary considerably in size, from that of a red blood corpuscle to several times the size of a red. The nucleus is large, generally round, but may be oval or bean-shaped. There is but a narrow rim of cytoplasm around the nucleus. The cytoplasm, with Wright's stain, takes a robin's-egg-blue tint and the nucleus stains a deep purple or purple-blue.

Granules are, as a rule, absent. Forms are seen, however, in which the cytoplasm contains from a few to a large number of pinkish but more generally blue granules.

The eosinophile is a large cell, resembling, in structure and size, the polynuclear neutrophile. With Wright's stain, the nuclei stain light blue or lilac, with an ill-defined intranuclear network. The large spherical or oval granules take a brilliant red eosin stain. The cytoplasm around the granules either takes no stain whatever or a pale blue tinge. This type of leucocyte occurs normally in very small numbers, and a number of fields may be gone over with the microscope before an eosinophile is found.

The mast cell is not commonly found in normal blood and you will probably not see it among the comparatively small number of white cells that you are required to count. The cell is about twice the diameter of the red cell, has a polymorphous nucleus of vague outlines, and a cytoplasm containing numerous very large granules taking a dark blue or blue-black stain.

VI. Estimation of Hæmoglobin.

A number of instruments have been devised for estimating the hæmoglobin of the blood. In most of these, for convenience of comparison, a scale of 100 is used, the 100 mark corresponding to a hæmoglobin content of 13.8 grams of hæmoglobin in 100 c.c. of blood. The instruments as a rule depend on a color comparison between the shed blood, with or without dilution, and a fixed scale of color to correspond to various dilutions. There is a certain degree of unavoidable error in the employment of any color test, which at times may be very high. A method which avoids the errors of the color comparisons is the estimation of the hæmoglobin from the specific gravity.

The simplest, least expensive, and most practical scheme of color test yet devised is that of Talqvist.

1. Talqvist's Hæmoglobinometer.—This consists of a paper scale of color shades varying from 10 to 100 per cent hæmoglobin and contained in a book of filter paper which is used for absorbing the specimens of blood whose percentage of hæmoglobin is to be estimated. The blood stain, undiluted, is compared with the hæmoglobin scale by reflected daylight until a shade is found to correspond to the tinge of the blood examined. For approximate clinical results this method is very satisfactory.

2. Dare's Hæmoglobinometer.—As in the method of Talqvist, undiluted blood is used. This is drawn by capillarity between two plates of glass, one of which is transparent, the other being translucent for diffusing the light used for illumination.

The color comparison is made with that of a glass disc which is revolved by means of a thumb screw so as to bring successive tints

into relation with the blood specimen being examined. Transmitted candle light is used for illumination. This instrument is more accurate than the Talqvist device, but is much less convenient and more expensive.

3. Hæmometer of v. Fleischl.—In this instrument the amount of hæmoglobin in a specimen of blood is estimated by comparing a stratum of diluted blood with a standard glass wedge

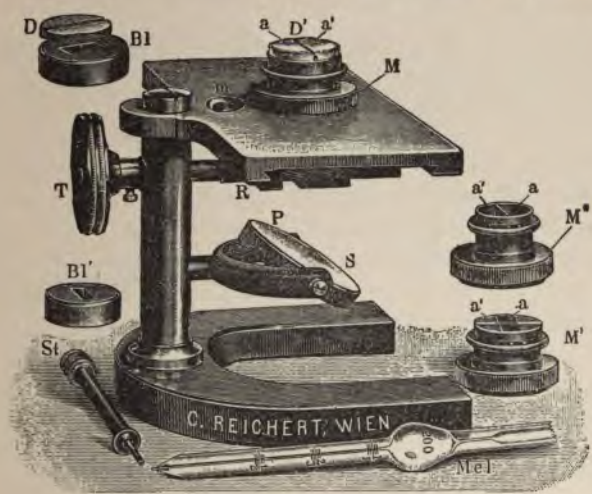

FIG. 25.—V. Fleischl's Hæmometer (modified by Miescher).

of uniform tint spectroscopically similar to that of the blood. This instrument has been recently modified and made more accurate by Miescher.

This modification of the instrument consists of a stand with a metal plate having a circular opening and a plaster mirror below (see Fig. 25), which serves to reflect the light through the colored wedge and diluted blood. Beneath the metal plate is a metal frame carrying the colored wedge alongside of which is a scale indicating the different percentages of hæmoglobin corresponding to the varying thicknesses of the wedge. This framework is

moved by the wheel (*T*), which fits into a rack on its lower surface. The scale may be read through a small opening (*m*) in the plate. Into the large circular opening of the plate, fits a cylindrical metal cell (*M*) with a glass bottom and divided by a thin metal partition into two equal parts. One of these halves lies over the wedge and is filled with distilled water. The other contains the solution of blood to be tested. The apparatus is usually supplied with three cells (*M*, *M'*, *M''*). Of these, the first two are used in estimating the hæmoglobin according to Miescher's modification of v. Fleischl's original method. These cells are furnished with a glass cover (*D*), having a groove which fits on the partition of the cell. Over this cover is placed a diaphragm (*Bl*), with a longitudinal slit, which permits the central part, only, of each side of the cell to be seen. The third cell (*M''*) is for use with the original method.

Procedure.—Blood from the wound is sucked up into the graduated pipette (*Mel*) until it reaches the mark $\frac{1}{2}$ or $\frac{2}{3}$ or $\frac{1}{1}$. A one-per-cent solution of sodium carbonate is then sucked in until the upper mark is reached. The pipette is then well shaken in order to mix the blood thoroughly. One-half of the two cells (*M*, *M'*), which are respectively 12 and 15 mm. high, are then filled with the mixture, the other half being filled with water. The cells should be completely filled. The cover glasses and diaphragm are then applied and the cells are ready for examination. Artificial light is employed. One of the cells is placed on the plate and the wheel (*T*) turned until the colors of the two halves exactly correspond. The result is then read off through the scale opening (*m*). This should be repeated several times with each of the cells and the average of the readings taken. The result obtained with the 12 mm. cell is to be multiplied by $\frac{5}{4}$ to bring it up to that of the larger.

Suppose the result of several readings to be as follows:

With the large cell (15 mm.) 54.00
With the small cell (12 mm.) 42.00

If the readings with the large cell are exactly correct, the reading with the smaller one should be 43.2, since $54 \times \frac{5}{4} = 43.2$. Or, if

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the reading with the small cell is correct, the reading with the large one should be 52.5, since $42 \times \frac{5}{4} = 52.5$. The mean of the two readings is taken as approximately correct.

Each instrument is supplied with a corrected scale of hæmoglobin values. Comparing the figure, obtained above, with the scale, it is found to correspond to a solution containing 400 milligrams of hæmoglobin in 1000 c.c. of solution. The dilution which was employed was 1 to 200, 1 to 300, or 1 to 400, according as to whether the pipette was filled to the mark $\frac{1}{2}$, $\frac{2}{3}$, or $\frac{1}{1}$. To find the actual amount of hæmoglobin in a given volume of blood, the result obtained would have to be mutliplied by 200, 300, or 400. In the example taken above with a dilution of 1 to 200 there would be 8 grams of hæmoglobin in 100 c.c. of blood.

4. Estimation of Specific Gravity.—It was first demonstrated by Hammerschlag that the specific gravity of blood bore a sufficient relation to the hæmoglobin content to make it of value in estimating the same. Variations in hæmoglobin ordinarily correspond quite closely to variations in specific gravity. With this in view Hammerschlag devised the following table showing the relation between specific-gravity changes and variations in hæmoglobin percentage:

Specific Gravity.	Per cent Hæmoglobin
1.033–1.035	25–30
1.035–1.038	30–35
1.038–1.040	35–40
1.040–1.045	40–45
1.045–1.048	45–55
1.048–1.050	55–65
1.050–1.053	65–70
1.053–1.055	70–75
1.055–1.057	75–85
1.057–1.060	85–95

The most convenient method for obtaining the specific gravity is through the use of two fluids with which the blood will not mix, one of a high density and the other of a lower density, such as chloroform and benzol.

The necessary apparatus consists of a urinometer jar, urinometer, a pipette of small calibre, glass rod, fine steel pens, bottle of chloroform, benzol, and a mixture of the two. The mixture should be approximately that of the estimated specific gravity of the blood to be tested.

A drop of blood is sucked up into the pipette. This is gently blown out below the surface of the mixture, care being taken to leave some blood in the pipette. It is well to have several drops in the mixture. If the specific gravity of the blood and that of the mixture is the same, the drop will float indifferently wherever placed. If the specific gravity of the mixture is greater than that of the blood drop, the latter will rise. If the mixture is lighter than the drop, the latter will sink. The density of the mixture may be increased by adding chloroform and decreased by adding benzol. The mixing of the two fluids is accomplished by careful stirring with a glass rod. When the density of the blood and that of the containing mixture is the same the specific gravity is taken by means of the urinometer.

The same chloroform-benzol mixture may be used repeatedly, if filtered after each test. Scrupulous cleanliness must be observed. Otherwise, particles of dust might adhere to the blood drop and thus cause an error. Care should also be taken to avoid the admixture of air with the drop of blood. The reading should, likewise, be taken as soon as possible, to avoid error through vaporization and through changes in the blood drop. If the hydrometer jar is not perfectly clean, the globule of blood is liable to adhere to the sides.

The advantages of this method of hæmoglobin determination are obvious. There is no delicate color comparison to be made. If proper precautions are taken, the experimental error is very small as compared with the color methods. The apparatus is simple and inexpensive and the technique is not difficult.

Estimate the hæmoglobin of a fellow-student by the several methods given above and compare results.

VII. Hæmoglobin and Its Derivatives.

1. Hæmoglobin Crystals.—Shake up some defibrinated blood with CO_2 gas; add ether, slowly, until the blood has become laked. Set in the cold for several days. Part of the hæmoglobin will have crystallized out and may be removed with a pipette and examined under the microscope.

Hæmoglobin of different animals crystallizes with varying facility. The hæmoglobin of man and of the herbivorous animals is very soluble and crystallizes with great difficulty. That of the rat and guinea-pig is much less soluble and therefore crystals are easily obtained.

With the blood of the rat, all that is needed is to take a drop of fresh blood, place it on the centre of a glass slide, add a drop of distilled water, and when the edges begin to dry, cover with a cover slip and examine under the microscope (Funke's method).

2. Hæmatin.—Hæmoglobin is composed of a pigment united with a proteid body which has erroneously, according to Schaefer, been called globin. The pigment may be separated from the proteid in the following manner:

To some defibrinated blood in a test tube add a few drops of KOH solution and heat gently. The solution assumes a greenish-red color. Now carefully neutralize by adding dilute HCl until the hæmatin is thrown down as a brownish precipitate.

The same result is attained through treatment of the blood with an acid and then neutralizing with an alkali. Hæmoglobin is therefore decomposed by acids and alkalies into pigment and albuminous compounds. All the iron is contained in the hæmatin.

3. Hæmatin Hydrochlorid (hæmin).—Place a very small drop of blood upon a glass slide. Mix with this a drop of glacial acetic acid. Heat to the boiling point over a small flame. Allow the fluid to evaporate and examine the residue under the microscope. Tiny reddish-brown prismatic crystals will be seen. These

are also known as Teichman's crystals, after their discoverer (see Fig. 26).

With dried blood as found in old blood stains, a small crystal of NaCl is added to the acetic acid before boiling.

This is a good test for the detection of blood stains, but does not identify the source of the blood.

4. Hæmatoporphyrin.—This is derived from the pigment portion of the hæmoglobin, hæmatin, by a splitting off of the iron radical.

Mix some dried blood in a test tube with concentrated sulphuric acid. Filter the resulting solution through asbestos. Divide the filtrate into three portions. To the first add an excess of distilled water. To the second add a weak solution of NaOH until the solution is slightly alkaline in reaction. To the third add an excess of acidulated alcohol.

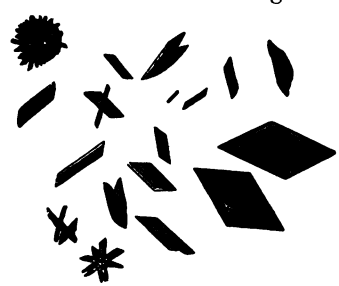

FIG. 26.—Hæmin Crystals. (Frey.)

The water in the first solution will precipitate the hæmatoporphyrin as a brown flocculent mass. The iron of the hæmatin unites with the acid to form a ferrous salt.

In the second tube the pigment goes into solution. In the third tube a solution is also formed. The alkaline solution is of a fine red tint changing to a violet in the presence of an excess of the reagent. The alcoholic solution has a purple color, changing to a bluish violet when strongly acidulated. These solutions, according to Schaefer, exhibit a magnificent red fluorescence, even when exceedingly dilute. This pigment occurs, in small amounts, in the normal urine, and in larger quantities in certain pathologic conditions, such as chronic sulphonal poisoning. It is also closely related to bilirubin, a bile pigment, and hæmatoidin (Virchow), which is found in old blood clots within the body.

VIII. Spectra of Hæmoglobin and Its Compounds.

A convenient instrument to use is the micro-spectroscope or spectroscopic ocular which fits into the tube of the microscope in place of the ordinary eyepiece.

First study the solar spectrum. With a narrow slit identify the Fraunhofer lines.

1. In a darkened room place a Bunsen flame in line with the reflecting mirror of the microscope so that the light from any body made luminous in the flame, will pass through the prisms of the spectroscope. Dip a platinum wire in a solution of sodium chlorid and heat in the flame. What is the nature of the resulting spectrum? Compare this with the spectrum of sunlight or the light from a Welsbach burner. What part of the spectrum is luminous? To what absorption band or Fraunhofer line does this correspond?

2. Repeat the observations with other metallic salts, such as strontium, potassium, barium, and copper.

3. Make a two-per-cent solution of eosin in water. Place this in a vial and clamp in the holder at the side of the eyepiece. Tilt the mirror until light passes through the solution and prism. Arrange the substage mirror for reflecting sunlight through the comparison prism so that the two spectra may be viewed side by side. The eosin absorbs certain parts of the spectrum. What are the absorption bands of eosin?

4. Oxy-hæmoglobin.—Fill the test vial with defibrinated blood diluted with ten volumes of distilled water. Place in the holder of the spectroscope and note what part of the spectrum is visible and what part has been absorbed. Increase the dilution until more and more of the spectrum becomes visible. Increase the dilution until the absorption bands can no longer be distinguished and the whole of the spectrum is visible.

What are the absorption bands of oxy-hæmoglobin? In what part of the spectrum do they occur? Make a drawing, comparing the absorption spectrum of oxy-hæmoglobin with the solar spectrum. (See text-book for pictures of various spectra.)

5. Reduced Hæmoglobin.—(*a*) Add to a dilution of defibrinated blood in which the two absorption bands distinctly show, a few drops of ammonium sulphid. Warm gently. The solution changes color, becoming purple or wine color. Examine the spectrum of this solution and compare it with that of oxy-hæmoglobin. Open the vial containing the reduced hæmoglobin solution, and shake it vigorously with air. Does the solution change color? Observe the spectrum. Has it changed? Is this change permanent or only temporary?

(*b*) Dilute the solution still further. Does the broad absorption band resolve into several or does it simply fade as the blood becomes more dilute?

6. CO-hæmoglobin.—(*a*) Through a dilute solution of defibrinated blood, showing the absorption bands of oxy-hæmoglobin, pass a stream of CO gas. Note the characteristic change in color from the bright scarlet of oxy-hæmoglobin to the cherry red of CO-hæmoglobin.

(*b*) Test the illuminating gas of the laboratory for carbon monoxide in this way.

(*c*) Observe the spectrum of the blood so treated. Does it differ markedly from that of oxy-hæmoglobin? Make a sketch showing the relations of the absorption bands.

(*d*) Add a reducing agent, as was done in obtaining reduced hæmoglobin. Is there any effect upon the color of the solution or upon its spectrum? What conclusion can you draw concerning the stability of this hæmoglobin compound? How does it compare in stability with oxy-hæmoglobin? Why is coal-gas poisoning so dangerous?

7. Methæmoglobin.—To a medium dilute solution of defibrinated blood, showing the two absorption bands of oxy-hæmoglobin, add a few drops of potassium-permanganate solution. Observe the spectrum. If the oxy-hæmoglobin bands still persist, add more of the permanganate and warm gently. Acidify the solution and look for the spectrum of methæmoglobin.

To the solution, add some reducing agent. Can reduced hæmoglobin be obtained from methæmoglobin?

8. Hæmatoporphyrin (hæmatin freed from iron).—Add concentrated sulphuric acid to some defibrinated blood in a test tube. Filter through asbestos. Examine the spectrum of this solution.

9. Hæmatin.—Prepare solutions of acid hæmatin, alkali hæmatin, ethereal solutions, acid alcohol solutions. Observe the spectra, comparing them with each other and with the spectra of the other hæmoglobin derivatives, studied above.

Drawings should be made of all the absorption spectra that you have seen, and these should be compared with the table of spectra in the text-book or hanging in the laboratory.

IX. Globulicidal Action of Serum.

1. Mix, in a small test tube, equal quantities of rabbit's blood and dog's blood serum. Let this stand for twenty to thirty minutes and then observe. Is there any change in the color of the mixture? Observe with reflected and transmitted light. Compare with blood that has been diluted with water. Place a drop on a slide and examine with the microscope. Compare this with a fresh sample of undiluted rabbit's blood.

2. Heat the dog's serum to 60° C. for ten to fifteen minutes and repeat experiment 1.

3. Repeat experiment 1, using rabbit's serum in place of dog's serum, and dog's blood in place of rabbit's blood. Do the same phenomena occur?

The sera of certain animals, when mixed with the blood of certain other species, cause a destruction of the cellular elements with a consequent escape of the hæmoglobin and its solution in the liquid portion of the mixture; or, in other words, laking occurs. This property is true for other cells than blood, so that, broadly, it is known as *cytolysis*. More specifically, in the case of blood, it is known as hæmolysis, and the serum causing such

changes is said to be lytic for those particular cells upon which it acts.

It has been found that a serum which is not normally lytic for the blood of a certain animal, may, through a so-called adaptive process, be made to acquire such properties. This may be demonstrated in the following series of experiments:

4. The serum of the guinea-pig is not normally lytic for the cells of rabbit's blood. Verify this statement by mixing equal parts of rabbit's blood and guinea-pig serum and examining, microscopically, for laking.

5. A guinea-pig is adapted for rabbit's serum in the following manner: 5 c.c. of rabbit's blood is injected into the abdominal cavity of a guinea-pig every third day until five injections have been given. The animal should be weighed, daily, and the temperature recorded. If the reaction following the injection be too severe, the quantity must be reduced. The quantity of the injection may then be increased providing that there is no decrease in weight or marked variation in temperature, and continued until ten injections are completed.

The pig is then bled, the blood allowed to clot, and the serum collected.

Bleed a rabbit. Collect the blood in a test tube containing three times the volume of 0.8-per-cent NaCl solution.

(*a*) Take a small portion of the diluted blood and add the same amount of the adapted pig's serum.

(*b*) To a second portion of rabbit's blood add equal parts of guinea-pig's serum which has not been adapted.

(*c*) To a third portion of blood, add some of the adapted serum which has been, previously, heated to 60° C. for thirty minutes.

(*d*) To a fourth portion of blood add equal parts of the adapted serum which has been heated and serum from a guinea-pig which has not been adapted.

Compare the dilute rabbit's blood, untreated, with tube (*a*), tubes (*b*), (*c*), and (*d*). Examine slides from each under the microscope.

Does laking occur in (*a*)? Does it occur in (*b*)? In (*c*)? In (*d*)? Are there any other phenomena observed aside from laking? What is the effect of heat upon the adapted serum? How can the combined action of heated adapted serum and unadapted serum be explained?

CHAPTER V.

CIRCULATION OF THE BLOOD.

I. Study of the Circulation in the Web of the Frog's Foot.

1. Inject a few drops of a one-per-cent solution of curare in the dorsal lymph sac of a frog. After fifteen minutes or a half hour tie the frog, face down, upon the frog-board. Spread the web, but not too tightly, over the opening in the board. Place the board on the stage of the microscope so that the opening in the board coincides with the opening of the stage. Study the circulation in the web with the low power first. If the circulation in the smaller vessels has stopped, the web is drawn too tight and must be somewhat more relaxed.

Note the direction of the flow from the larger vessels into the smaller ones and from the smaller toward the larger. Can you make out a pulsation in any of the vessels? If so, in which ones? What is the direction of the flow in the pulsating vessels, from large vessel into branches or from branches into large vessel? What is the speed of flow in the large vessels as compared with the smaller ones? Can you make out the outlines of the corpuscles in any of the vessels? Can you distinguish more than one kind of corpuscle?

2. Examine now with a higher power, so that the corpuscles may be distinctly seen. Select a small vessel where the flow is not so rapid and note the position and speed of movement of the red and white corpuscles in the blood stream.

As determined by the movement of the corpuscles, in what part of the stream is the speed of flow greatest? Explain.

How do the red and white corpuscles compare in numbers?

In the smaller and narrower vessels where only one corpuscle at a time can get through, note the adaptability of the red cells, through their elasticity, to the varying calibre of the vessel.

3. The Migration of the Leucocytes.—Dip the point of a pin into strong acetic acid. Touch the web with the acidulated pin point. Note the ensuing effect upon the circulation through the web as a whole and particularly upon that part in the immediate vicinity of the irritant.

Examine a small vessel with the high power. How does the relative number of reds and whites compare with the relative number in the non-irritated web?

Find a portion of the web free from pigment, where a capillary may be seen whose walls are distinctly visible. Pick out some leucocyte lying against the wall of the vessel and observe it closely. If a good field has been selected, the corpuscle will be seen to make its way, gradually, through the capillary wall. Make a series of sketches showing the progress of the corpuscle through the wall until it is entirely outside of the vessel. After a time many white cells will be found outside of the vessels in the surrounding tissue. This process is known as the migration of the leucocytes and occurs in other inflammatory conditions where the specific irritant is some micro-organism or bacterial toxin.

Where the resulting inflammation is more severe, the vessel walls may so change as to allow the passage of the red cells as well as that of the whites. The white cells, however, pass through the vessel wall by means of their amœboid movement, while the reds, in their passage, are entirely passive, being forced through by the pressure of the blood in the vessel.

4. Repeat the observation of the capillary circulation, using the frog's mesentery instead of the web of the foot.

The mesentery is so sensitive that simple exposure to the air acts as a sufficient irritant to cause an exhibition of all the phenomena of inflammation.

Make a drawing of the capillary circulation as seen in the mesentery and in the web of the frog's foot.

II. Direct Observation of the Action of the Frog's Heart.

Pith the frog used in the previous experiment or, if necessary, take a fresh frog. Lay the frog on its back upon the cork frog-board, spreading the fore legs out and pinning them to the board. Do the same to the hind legs. Make a median incision through the skin of the thorax, crossing this transversely with an incision at the level of the fore limbs. Lay back the flaps of skin and, with small

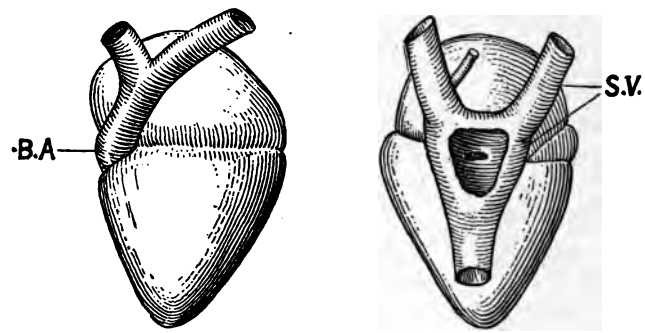

Fig. 27.—Frog's Heart. *V*, Ventral view; *D*, dorsal view. *B.A*, Bulbus arteriosus; *S.V*, sinus venosus.

but strong scissors, continue the median incision through the thoracic muscles and sternum. Be careful to keep the lower blade of the scissors snugly against the inner surface of the sternum in order to avoid injury to the pericardium and heart. Hook back the divided sternum so as to expose the heart in the pericardium.

Note the relations of the pericardium to the heart and great vessels and to the surrounding viscera. Keep the preparation moist with 0.6-per-cent NaCl solution.

Before opening the pericardium, note the rate of the heart beat, counting the number of beats in a minute.

Now open the pericardium so as to obtain a better view of the different pulsating portions of the heart and make the following observations:

(*a*) The different contracting parts (see Fig. 27) and the sequence of contraction.

(*b*) The change in form of the different contracting parts.

(*c*) The change in color of the different parts during contraction as compared with relaxation.

(*d*) The duration of the systolic period as compared with the diastolic phase.

(*e*) The change of position of the heart, as a whole, with each systole.

(*f*) Gently grasp the ventricle between the thumb and first finger and note the hardening of the muscle with each systole.

(*g*) Now carefully excise the heart, including all its pulsating parts, *i.e.*, the sinus venosus with the large veins which empty into it, and the bulbus arteriosus with pieces of the arteries into which it branches. Place the excised heart in a shallow dish or a watch glass containing 0.6-per-cent NaCl solution. Does the heart continue to beat? Is the normal sequence of contraction of the different parts still continued? What conclusion can you draw from this observation concerning the dependence of the heart beat upon the central nervous system?

Count the number of heart beats per minute and compare with the rate of pulsation before excision.

(*h*) Warm the heart above the surrounding room temperature, by holding the containing vessel in the hand. How is the beat affected?

Float the watch glass on cold water or set it on some snow. How is the rate of pulsation affected compared with the normal and with that of the warmed heart?

(*i*) Using the same heart or a fresh one if necessary, cut off the sinus from its connection with the auricle. Does the sinus continue to beat? Do the auricles and the ventricle continue to beat? If so, is there any difference in rate of pulsation between the different parts?

(*j*) Sever the auricles from the ventricle by an incision through the auriculo-ventricular groove. Note results as before.

(*k*) Separate the auricles from each other. Record results.

(*l*) If the ventricle has stopped beating, see if it will still respond to mechanical or electrical stimuli.

Draw conclusions from the above observations concerning the mechanism of the heart-beat.

III. Graphic Record of the Frog's Heart-beat.

Pith a frog. Pin, back down, on the frog board. Expose the heart as in the previous experiment. The heart may be connected with the heart lever by one of two ways.

1. The Suspension Method.—Make a small hook out of a bent pin. Pass this through the tip of the ventricle, avoiding, if possible, piercing the cavity of the ventricle. Attach this hook, by means of a fine thread, to the short arm of the heart lever, placing sufficient counterpoise upon the long arm to balance the weight of the thread and pin and to raise the heart slightly from its bed. Apply the writing point of the lever to the smoked paper of a medium fast drum. Also, beneath the heart record, make a time tracing in seconds.

The auricular beat may be recorded separately, at the same time, by attaching the auricle, in the same way, to another lever which may be adjusted to write under or over the ventricle lever.

2. Direct Transmission Method.—An upright, made of bamboo or some other light material, is attached to the long arm of the lever nearer to or farther from the axis, depending upon the magnification desired. The lower end of the upright is supplied with a cork foot which may be made to rest upon the auricle or ventricle. Every movement of the heart chamber upon which the foot of the upright rests will be transmitted to the long arm of the lever and will be recorded upon the revolving drum.

By the use of either one of these methods record the contractions of the ventricles and auricles, noting the rate of pulsation and the duration of each phase. Also note the form of the curve obtained during systole. Compare systole with diastole.

With the suspension method the ventricular record will prob-

ably include also a tracing of the auricular contraction and, if the counterpoise is delicate enough, the sinus may likewise be included.

Note the relation and sequence of contraction of sinus, auricles, and ventricle.

With direct transmission, the relations of auricular pulsation to ventricular systole and diastole may be observed by placing the foot of the writing lever upon the auriculo-ventricular groove.

The relation of ventricle to bulbus contraction may be shown in the same way, by adjusting the foot of the lever to rest partly upon the ventricle and partly upon the bulbus.

Make careful record of all observations and interpretations of results, marking all data necessary for identification upon the tracing.

Compare your results with those of other students and explain differences.

What part of the tracing is due to errors of adjustment of, and inertia of, the apparatus?

IV. Influence of Temperature upon the Beat of the Frog's Heart.

Use the same heart as in the previous experiment, or, if this is not vigorous enough, make a fresh preparation.

Record the beat of the ventricle, taking a time tracing in seconds or half-seconds in order to determine the rate of pulsation.

(*a*) Determine the frequency of the beat at the room temperature, making a note of the temperature of the room.

(*b*) Bathe the heart for several minutes with 0.6-per-cent NaCl solution warmed to 37° C. Allow the drum to revolve again at the same speed as before, recording the second tracing under the first one so that a comparison may easily be made.

(*c*) Bathe the heart again with salt solution at the room temperature. Continue the bathing with salt solution cooled in an ice bath to 5° C. Make another record, under the first two, together with a time tracing.

How do (*a*), (*b*), and (*c*) compare in the number of beats per minute?

How do the contraction curves compare with each other?

How does the time ocupied by the systolic phase compare with the time occupied by the diastolic phase in each tracing?

When the frequency of the heart-beat is increased, is the time occupied by systole and that of diastole decreased in the same ratio?

If not, which is shortened the more, systole or diastole?

V. Dissection of the Extrinsic Cardiac Nerves of the Frog.

With a preserved or fresh dead frog, expose the heart as before. Carefully cut away the sternum and muscles of the thorax. Locate the glosso-pharyngeal and hypoglossal nerves (see Fig. 28). Also

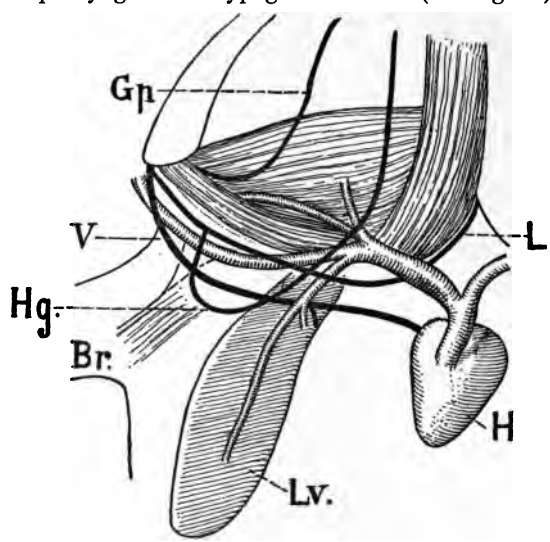

Fig. 28.—Extrinsic Cardiac Nerves of Frog. *V*, Vago-sympathetic; *Gp*, glosso-pharyngeal nerve; *Hg*, hypoglossal nerve; *Br*, brachial plexus; *L*, laryngeal nerve; *H*, heart; *Lv*, lung.

note the petrohyoid muscle. Along the lower border of this muscle and lying between the loops of the above-mentioned nerves, are two fine grayish fibres. These are, the lower one the vagus trunk,

containing both accelerator and inhibitor fibres, and the upper one the laryngeal branch of the vagus.

Trace the cardiac branch of the vagus trunk to its distribution in the heart. This trunk as thus exposed in the thorax is really a combined nerve, being formed by the junction of the vagus proper and the cardiac branches of the sympathetic.

VI. Effect of Stimulation of the Vago-Sympathetic Trunk upon the Heart-beat.

Pith a frog. Carefully expose the heart and isolate the vago-sympathetic trunk. Place this upon fine platinum electrodes, avoiding, so far as possible, contact with other nerves or the surrounding tissues.

Connect the ventricle, by the suspension method, with a light lever made to write upon a medium fast drum.

(*a*) Set up the inductorium for weak tetanizing induction shocks and connect the secondary with the vagus electrodes. Arrange a time-marker writing quarter-seconds to trace beneath the heart-beat record.

While the ventricular tracing is being taken, stimulate the vagus with weak induction shocks for ten seconds. Is there any change in the rate or strength of the heart-beat? If no appreciable effect is produced, increase the strength of the current slightly, noting the distance of the secondary from the primary, and repeat the stimulation of the nerve.

(*b*) Increase the strength of the stimulus, noting the effect on the heart-beat with each increase, until strong stimulation is employed.

What is the effect of weak stimulation of the nerve? Of stronger stimulation? Of the strongest stimulation which you applied?

What is the after-effect upon the heart-beat, following the cessation of the stimulation?

(*c*) Apply a strong stimulus to the nerve and continue this for a minute. Does inhibition continue during the entire time of the application of the stimulus?

Explain the difference in the effect of strong and weak stimulation.

(*d*) *Effect of Stimulating Vagus Terminals.*—Using the same heart, stimulate behind, between the auricles and the sinus venosus. Compare these results with those of (*a*), (*b*), and (*c*).

VII. Reflex Inhibition of the Frog's Heart.

Prepare a fresh frog. Do not pith, but anæsthetize, lightly, with ether. Expose one sciatic nerve as well as the heart. No tracing need be made. The beat may be studied by direct observation.

(*a*) Count the number of beats in ten seconds; in one minute. With a scalpel handle, gently tap the abdomen in the stomach region for five seconds. During and after the tapping, observe and count the number of heart-beats. What is the effect of the tapping upon the beat of the heart? Is it accelerated or inhibited?

(*b*) Introduce into the stomach, by way of the mouth and œsophagus, a pair of shielded electrodes. In this way stimulate the stomach with a medium strong current and note results in relation to the heart-beat.

(*c*) Cut the sciatic nerve. Is there any effect upon the heart-beat? Stimulate its central end with a medium strong current. What is the effect upon the heart-beat?

(*d*) Cut both vagus nerves. Can reflex inhibition now be obtained?

VIII. Effect of Drugs.

1. Atropine.—Pith a frog. Expose the heart and vago-sympathetic nerve. Place a pair of small electrodes under the nerve and connect these with the inductorium arranged for medium strong tetanizing current. Connect the ventricle with the heart lever for recording on a medium fast drum. Take a normal tracing, before and after bathing the heart with physiological salt solution. Take a time tracing for comparison. Stimulate the nerve with the tetanizing current for several seconds. There should be inhibition of the heart-beat.

Wait until the after-effects of the nerve stimulation have disap-

peared and then bathe the heart in a 0.2-per-cent solution of atropine sulphate, first confining the application of the drug, so far as possible, to the sinus venosus. Is the heart-beat altered in any way?

Now repeat the stimulation of the nerve. What is the effect on the heart-beat? Is there acceleration or inhibition? Compare this with the tracing obtained before the application of the drug.

Stimulate the vagus at the sinus. Is any inhibitory effect produced? What effect, then, has atropine upon the extrinsic nervous mechanism of the heart?

Continue the application of the drug until its effect upon the heart muscle becomes manifest. Wash repeatedly with physiological salt solution. Does the heart recover from the effects of the atropine? Repeat the stimulation of the nerve. Is inhibition again finally brought about?

2. Cocaine.—If the heart used in experiment 1 has recovered it may be used again for this experiment. Otherwise, a new preparation will have to be made.

The heart is arranged for recording as before and the vagus nerve, for stimulation. A control tracing is made of the heart-beat with and without stimulation of the nerve.

While the tracing is being taken, allow a few drops of a 0.2-per-cent solution of cocaine in 0.6-per-cent salt solution to fall upon the heart. Note the effect upon the heart-beat. Is there any disturbance of co-ordination?

After any irregularity in the beat that may have occurred has disappeared, stimulate the vago-sympathetic nerve. Is any inhibitory effect produced?

Continue the application of the cocaine solution until the ventricle ceases to beat. Note the changes in the heart's action as the cocaine effect is continued and increased.

3. Pilocarpine.—In a pithed frog, make a heart preparation for recording, and expose the vago-sympathetic nerve for stimulating. Take first a tracing of the beat before the addition of the drug. Make another tracing during stimulation of the nerve

Now bathe the heart in a ten-per-cent solution of pilocarpine hydrochlorate. What is the effect upon the beat?

Stimulate the nerve. Is there any inhibitory effect? If so, add more of the pilocarpine solution. Stimulate the nerve again. If there is no inhibition, is there any acceleration?

After the heart is again beating slowly, add a few drops of weak atropine solution. What is the result? Add more pilocarpine. Does the atropine effect disappear and the pilocarpine effect reappear? Repeat this alternate treatment with pilocarpine and atropine, several times. Explain the action of pilocarpine from the observations thus made. Upon what does the antagonism of the two alkaloids, pilocarpine and atropine, depend?

4. Muscarin.—Expose the heart and vago-sympathetic nerve of a pithed frog. Take a tracing of the normal heart-beat. Next, take a tracing of the beat while stimulating the nerve.

Bathe the heart in a ten-per-cent muscarin solution. Compare the effect upon the beat with the pilocarpine effect and with the atropine effect. Stimulate the nerve. Is there any result? Add a few drops of atropine solution. What is the effect? Explain.

5. Digitalin.—Prepare a frog as before. Take a tracing of the normal beat and of the beat during vagus stimulation. Apply to the heart a few drops of a saturated solution of digitalin, while a tracing is being taken. Carefully note the effect upon the strength of the beat, the rate of beat, and the change in systole and diastole.

Continue the application of the alkaloid until the heart ceases to beat. Does the heart stand still in systole or diastole?

6. Nicotine.—Expose heart and vagus nerve in a pithed frog. Make a record of the beat before and during stimulation of the nerve. Note the character of the normal contraction. Now bathe the heart with a 0.1-per-cent solution of nicotine in physiological saline. Continue the tracing during the application of the drug. What is the effect upon the frequence and strength of the heart-beat?

While the heart is still under the influence of the drug, stimulate

the vago-sympathetic nerve with a strong tetanizing current. Is there any inhibition of the heart-beat?

Stimulate the sinus while the nicotine effect is still manifest. Is there any inhibition of the heart-beat? How can you explain the difference in result between stimulation of the nerve trunk and stimulation of the point of transfer in the sinus? Upon what part of the nervous mechanism does the nicotine act?

Now bathe the heart with a stronger nicotine solution (one per cent). Note the effect. To what is the effect due?

Paint the heart with the pure alkaloid. In which phase does the heart's action cease?

7. Ether.—Expose the heart of a pithed frog. Connect with cardiograph lever and take a tracing of the normal beat. Shake up some ether with physiological salt solution and bathe the heart with it, while a tracing is being taken. Observe the effect upon the frequency and strength of the beat. Continue the application of the drug and the observation of the beat of the heart.

Wash the heart with undiluted ether until the beat grows feebler and finally nearly ceases. Wash with physiological saline.

Can the beats be restored? Repeat the bathing with strong ether until the beats have again nearly ceased and then allow a few drops of a 1 to 10,000 adrenalin solution to flow on the heart. Result?

8. Chloroform.—A frog is pithed, as before, and the heart exposed. A tracing of the normal beat is taken as a control. A time tracing, in seconds, is also taken.

Physiological saline is shaken to saturation with chloroform. While the tracing is continued, the heart is bathed with this mixture. What is the primary effect of the application of the chloroform?

Continue the application of the drug. How does the continuation of the chloroform application affect the heart-beat?

Discontinue the application of the drug and wash the heart with physiological salt solution. Does the heart recover from the effects of the chloroform?

Bathe again with chloroform, this time undiluted. Continue until the heart stops beating. In what phase does the stillstand occur? How does the chloroform effect compare with that of ether? How does the heart-beat change in frequency? In strength? In length of diastole and systole? To which is the heart more susceptible, chloroform or ether?

9. Suprarenal Extract (*Adrenalin*).—Take a tracing of a normally beating frog's heart. Make up a 1 to 10,000 solution of adrenalin chlorid in physiological saline. Bathe the heart with this solution and record results. What is the effect on the strength and frequency of the heart-beat? Upon systole and diastole?

Place the web of the frog's foot under the microscope. Locate certain vessels whose outlines are quite distinct. Add a few drops of the adrenalin solution to the web and note the effect upon the calibre of the vessels.

IX. Perfusion of Frog's Heart.

Pith a frog and expose the heart. Excise the heart, including the sinus venosus. With sharp-pointed scissors make an opening in the auricles. Introduce, through this opening, Kronecker's perfusion cannula into the ventricle. Secure the cannula by means of a ligature tied above the base of the ventricle. Connect one limb of the cannula with the perfusion tube and the other with the small frog's-heart manometer (see Fig. 29).

Allow the heart to hang in the normal saline bath. Connect this with one pole of a dry cell. Connect the other pole with the binding post on the cannula. Interpose a key in the circuit. The heart may stop beating for several minutes after the cannula is tied in. The beats, however, will generally begin spontaneously after a short time. If not, closing the key of the constant current, and thus stimulating the heart, will probably be sufficient to bring about rhythmical pulsations. Simple distention of the ventricle with the perfusing fluid may be enough of a stimulus.

Fill one perfusion tube with 0.6-per-cent NaCl solution and the

other with defibrinated rabbit's or dog's blood diluted with equal volumes of 0.6-per-cent NaCl solution.

Perfuse with the physiological salt solution. From time to time, close the outlet tube of the manometer and record the changes in ventricular pressure on a revolving drum of medium speed. Continue the perfusion until the rhythmical beat ceases.

Now perfuse with the diluted defibrinated blood. Do the rhythmical pulsations again begin?

Repeat with a fresh heart preparation, using the diluted blood from the start. How long are rhythmical contractions continued with the blood mixture as compared with the NaCl solution alone? Record the pressure changes as before.

Fill one perfusion tube with physiological saline, saturated with ether. Perfuse the heart with this solution. Record the pressure changes with the manometer. Compare the effect of ether perfusion with that obtained through the direct application of the drug.

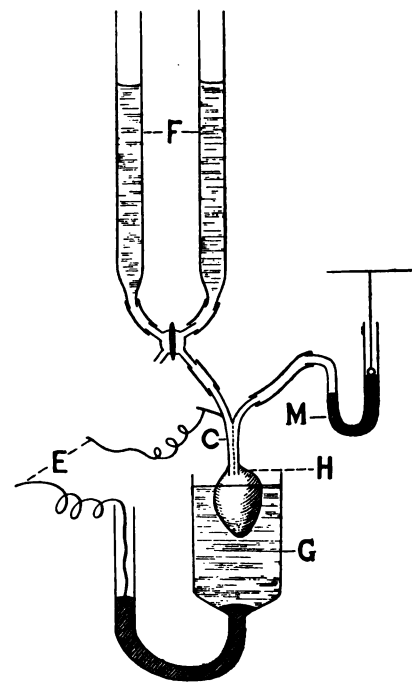

FIG. 29. — Frog's Heart Perfusion Apparatus. (Kronecker.) *H*, Heart; *C*, cannula; *G*, glass containing physiological salt solution; *E*, wires of constant current; *F*, perfusion flasks; *M*, manometer.

Change the perfusion to diluted blood, thoroughly washing out the ether solution. Do the heart-beats return to the normal?

If so, replace the ether solution with one of chloroform and perfuse with the latter. Record the movements of the manometer needle. Compare the chloroform effect with that of the ether and with that obtained through the application of chloroform to the outside of the heart.

X. The Action of Certain Salts on the Heart Muscle.

Expose and remove the heart of a turtle. Make a ventricular muscle preparation as follows: Make two cuts through the ventricle, a little below and parallel with the auriculo-ventricular groove. The cuts thould be about 3 mm. apart.

The ring of ventricle thus obtained is cut through at opposite sides, so that two pieces of nearly uniform length and thickness are obtained.

Attach one end of each strip, by means of a fine silk thread, to the short arm of a light counterpoised muscle lever. The other end is attached to one limb of a glass rod, bent at right angles, the other limb of which is held stationary. The lever is adjusted to the surface of a slowly revolving drum. Each contraction, therefore, of the muscle strip is recorded. By the time the preparation is complete, the muscle will, probably, have ceased beating.

1. Sodium.—(*a*) Let the preparation dip in a beaker containing a 0.7-per-cent solution of NaCl. How long before rhythmic contractions of the muscle strip begin? How long do they continue? What are the rate and character of the contractions? Does sodium act as a stimulus to contraction? Is an isotonic sodium solution sufficient to maintain rhythmical contractions?

(*b*) After contractions have ceased in the strip immersed in the sodium solution, remove the strip, blot off the excess of the solution with filter paper, and immerse in another beaker, containing an isotonic calcium-chlorid solution (about one per cent).

(*c*) Do the contractions reappear in this solution? If not, immerse again in the sodium-chlorid solution. Do contractions now appear?

2. Calcium.—Immerse another strip of muscle in calcium-

chlorid solution, without previous immersion in the sodium-chlorid solution. Is the muscle stimulated to pulsate? Does calcium act as a stimulus to ventricular contraction?

3. Combined Action of Sodium and Calcium.—To a 0.7-per-cent solution of sodium chlorid in a beaker add one-tenth of the volume of calcium chlorid. Immerse a fresh muscle preparation in this solution. How does the length of time during which contractions are maintained compare with that in which sodium chlorid alone was used? What is the character of the individual contractions?

4. Potassium.—Immerse a strip of ventricular muscle in a 0.9-per-cent potassium-chlorid solution which is nearly isotonic with 0.7 per cent NaCl. Are rhythmical contractions brought about?

5. Combined Action of Sodium, Calcium, and Potassium.—Immerse a strip of ventricular muscle in a solution of sodium chlorid 0.7 per cent, calcium chlorid 0.025 per cent, and potassium chlorid 0.025 per cent (slightly modified Ringer's solution).

Record the contractions upon a very slowly moving drum. How long will rhythmical contractions continue? How do they compare with those obtained from the muscle treated with 0.7 per cent NaCl alone, and with those from the muscle immersed in a combination of Na and K?

XI. Stannius' Experiment.

Pith a frog. Carefully expose the heart. Tie the frænum, the partition of pericardium attached to the dorsal aspect of the ventricle, and use the ligature as a guide. Pass a thread around the junction of the sinus with the auricles and tie snugly (see 1, Fig. 30).

The sinus continues to beat. The auricles and ventricle stop beating. Now tie a second ligature around the heart at the auriculo-ventricular groove. The ventricle again begins to beat. The auricles will probably remain quiescent.

Tie a third ligature about the middle of the auricles, at line 2, as

indicated in the figure. The auricles will again begin to beat, but at a different rate from that of the ventricle.

Tie a fourth ligature about the base of the ventricle as indicated by line 4, Fig. 30. The ventricle will again cease to beat.

XII. Maximal Response of Heart Muscle to Minimal Stimulus.

1. Stop the rhythmical contraction of a frog's heart by applying the first Stannius ligature.

Set up inductorium for single induction shocks. Connect tip of ventricle with the cardiograph lever. Arrange the drum for movement by hand. With the secondary coil removed as far as possible from the primary, apply the electrodes to the ventricle and break the primary circuit. No contraction will probably occur. Move the secondary nearer the primary and repeat the breaking of the circuit, at intervals of ten seconds, until a stimulus is found which will cause the ventricle to contract.

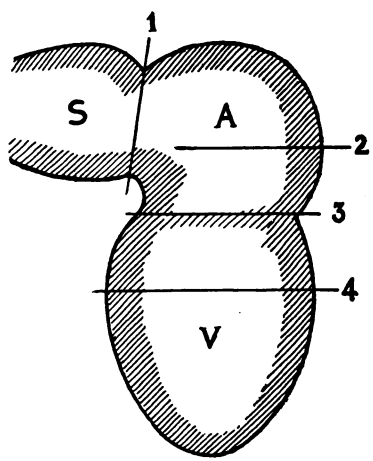

Fig. 30.—Schematic Frog's Heart, to Show Application of the Stannius Ligatures. 1, Between sinus (*S*) and auricles (*A*); 2, middle of auricles; 3, between auricle (*A*) and ventricle (*V*), at auriculo-ventricular groove; 4, about base of ventricle, below groove.

Move the drum slightly, increase the strength of stimulus, and record again. Repeat with stronger and stronger stimuli.

The contraction in response to the strongest stimulus is no greater than the one in response to the weakest stimulus that will cause a contraction. The heart muscle, therefore, responds to a minimal stimulus by a maximal contraction.

Compare this with the response of skeletal muscle to stimuli of various strengths.

XIII. Refractory Period.

Connect a frog's heart with the cardiograph lever. Let this record upon a medium rapid drum. Arrange a signal magnet in the primary circuit, with the point of its writing lever placed directly under the point of the cardiograph lever.

Stimulate the ventricle at intervals of ten or fifteen seconds, with maximal make or break shocks from the inductorium. Attempt to stimulate each time at a different part of the heart's cycle.

Some of the stimuli will be accompanied by an extra-contraction; others will have no effect so far as calling forth an extra-ventricular contraction is concerned. The first stimuli were applied during the irritable stage of the heart muscle. The second were applied during its *refractory period.* Where an extra-contraction occurs, it is followed by a pause, known as the *compensatory pause,* since it generally restores the rhythm which prevailed before the extra-contraction occurred.

Systematically stimulate the ventricle at different periods of the cycle and note your results on the drum, thus mapping out the limits of the refractory period.

XIV. Dissection of Mammalian Heart.

Open the thorax of a dead rabbit. Note the position of the heart in the pericardium and its relation to the surrounding viscera. Note the reflections of the pericardium over the great vessels which enter and leave the heart. Open the pericardium and note the position of the heart in the thorax. What part of the heart is in closest relation to the chest wall? What is the position of the right and left ventricles in relation to the anterior thoracic wall? In relation to the diaphragm? Note the number of auricles and ventricles in comparison with the frog's heart. Also note the more distinct differentiation of the pulmonary system in the mammal as compared with the frog.

By means of a V-shaped incision through the walls of each ventricle, with the apex of the V toward the apex of the ventricle, open the right and left ventricles. Note the openings between the auricles and ventricles and between the ventricles and large arteries. Note the shape and attachments of the auriculo-ventricular valves, the chordæ tendineæ and papillary muscles. Compare these with the valves guarding the openings of the large vessels, the semilunar valves. Determine the direction of opening of the various valves.

XV. Dissection and Relations of the Extrinsic Cardiac Nerves.

This should first be done upon the dead animal. Make a median incision in the neck of a rabbit, through the skin and superficial fascia. Continue the incision down to the trachea, which is used as a guide. Separate the muscles from either side of the trachea and pull them to one side. Beneath the sterno-mastoid muscle the carotid artery and the large nerve trunks running along with it will now be exposed. Immediately behind the artery lies the vagus nerve trunk. This nerve contains, among a number of other fibres, inhibitory fibres for the heart. A somewhat smaller nerve is seen behind and to the inner side of the artery. This is the cervical sympathetic. Next to this, in the rabbit, is a small nerve, the depressor. Opposite the larynx, the superior laryngeal branch joins the main vagus trunk. Here also the depressor nerve joins the superior laryngeal and enters the main trunk with it or splits into two branches, one joining the vagus and the other the superior laryngeal (see Fig. 31).

XVI. Direct Observation of the Pulsating Mammalian Heart.

Inject under the skin of a rabbit ½ grain of morphine sulphate. Lightly anæsthetize with ether. Expose the trachea through a median incision in the neck. The rabbit should be tied, back down, upon the rabbit-board, the fore limbs being brought down to the

sides of the thorax and secured. The neck should be well stretched and secured in the head-holder. A simple way of doing this is to pass a short thick glass rod or wire nail through the mouth, behind the teeth, and tie about the jaws and to the board behind the head.

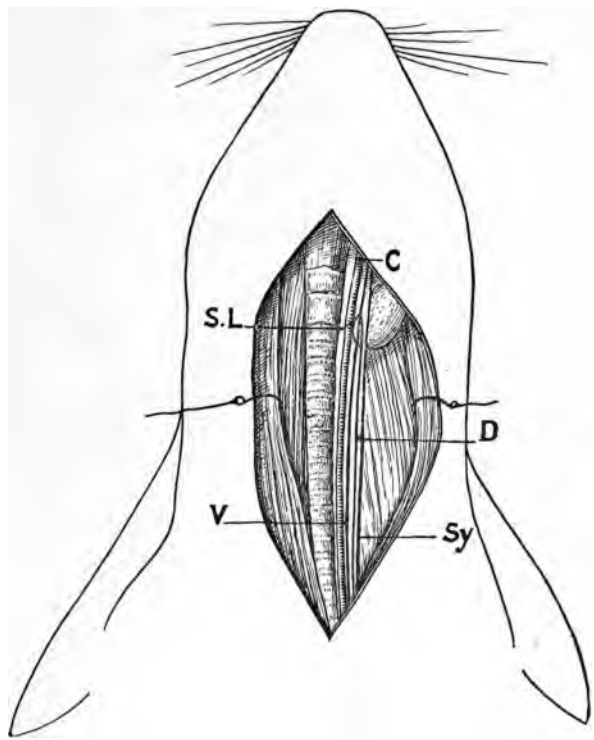

FIG. 31.—Extrinsic Cardiac Nerves of Rabbit. *C*, Carotid artery; *V*, vagus nerve; *D*, depressor nerve; *Sy*, sympathetic nerve; *S.L*, superior laryngeal nerve.

Open the trachea by a transverse incision. Introduce and tie in the tracheal cannula. Connect this with some form of artificial respiratory apparatus and start artificial respiration at about the same rate that the rabbit was breathing before.

Make a median incision through the skin of the thorax down to the sternum and as far down as its lower end. With heavy scissors

or bone scissors specially made for the purpose, cut through the sternum, keeping the lower blade of the scissors closely applied to the under side of the sternum. With care, the internal mammary artery will probably be avoided. If it is cut, it must be secured with artery forceps and tied.

The artificial respiration should be stopped during the operation of opening the thorax, in order to avoid injuring the lungs. Start the artificial respiration again. Pull the divided thorax apart and expose the pulsating heart in the pericardium. Note its relations to the lungs during inspiration and expiration. Stop the respiration for a moment and open the pericardium. Note the character and sequence of contraction of the various chambers. Note the change of position of the heart with each beat. Compare ventricular systole with diastole. Note the coronary arteries and veins on the surface of the heart. How does the method of nutrition of the mammalian heart compare with that of the frog? Feel the heart between the thumb and finger. Note the hardening of the ventricle with each systole and its softening with each diastole.

With artificial respiration stopped, note the change in the rate of the heart-beat as asphyxia continues, also the distention of the right ventricle. Also note the change in color of the blood. Begin respirations again and note the recovery of the heart and the change in the color of the blood.

XVII. Stimulation of Extrinsic Cardiac Nerves.

1. In the same rabbit used in the previous experiment, expose and isolate the vagus and depressor nerves. Tie both nerves between ligatures. Cut between the ligatures. Stimulate the distal end of the vagus with a strong tetanizing current and observe the heart's action. Stimulate the central end of the divided depressor and observe the effect upon the heart-beat. Stimulate the distal end of the depressor. Is there any change in the beat of the heart?

Trace the cervical sympathetic down into the thorax to the stellate ganglion. Place this upon the electrodes and stimulate with a medium strong tetanizing current. What is the effect

upon the heart-beat? Compare this with the strong vagus stimulation.

Stimulate the ventricle directly with a tetanizing current and note the change in the character of the contractions. Compare the feel of the heart in this condition with that of the normally beating heart. After the cessation of the exciting cause of the fibrillary contractions, the rabbit's heart will again pulsate normally. The heart of the dog will continue to fibrillate until death, unless it is removed and perfused with defibrinated blood or saline solution.

Excise the heart. Does it still continue to beat? Sever the ventricles from the auricles by a cut below the auriculo-ventricular groove. Do the severed parts continue to pulsate? If the ventricles have stopped, are they still irritable to mechanical stimuli? Sever the auricles from each other. Do they still continue to beat?

XVIII. Action of the Heart Valves.

The following simple scheme may be used to demonstrate the action of the semilunar valves (see Fig. 32). A dog's heart or a

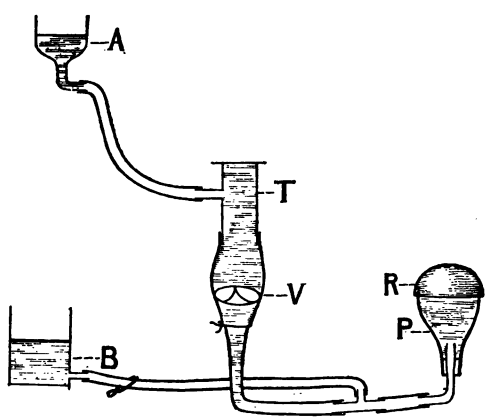

Fig. 32. —Apparatus to Show Action of Semilunar Valves. (Description in text.)

fresh pig's heart may be used. A tube, *T*, with a smoothly cut end over which is cemented a flat piece of glass, is connected through

a sidepiece to a pressure bottle, *A*. The open end of the tube is inserted into the aorta or pulmonary artery, above the semilunar valves, and tied in. The ventricle behind the valves is cut away, enough being left for the insertion and securing with a snugly tied ligature of another glass tube which is connected through rubber tubing with another pressure bottle, *P*, used for exerting pressure on the ventricular side of the valves. Pressure bottle *B* is used for the filling of *P*, and can be clamped off when this has been accomplished.

Bottle *P* has had the bottom removed, and in place of it a heavy rubber dam has been substituted. By raising or lowering bottle *A*, the pressure on the arterial side of the valves may be increased or decreased. The movement of the valves may be observed through the glass plate of tube *T*.

Make sudden pressure with the hand on the rubber membrane of bottle *P*. Note the opening of the valve flaps. In which direction do they open? Note their closure when the pressure on the arterial side is greater than that on the ventricular side. Increase the arterial pressure by elevating the bottle *A*. Note the increased pressure needed at *P* to open the valves. What prevents the valves from being forced back into the ventricle?

The same scheme may be utilized for showing the action of the auriculo-ventricular valves by tying tube *T* into the auricle, and the tube connected with bottle *P* into the ventricle, with the aorta clamped off.

XIX. Mechanics of the Circulation as Studied with an Artificial Schema.

Set up an artificial scheme of the circulation as shown in Fig. 33. *H* represents a Davidson's syringe having an inlet and an outlet valve which correspond to the valves between the auricles and ventricles and between the ventricles and arteries, respectively. The fluid is, therefore, allowed to flow in one direction only. *A* represents arteries, *C* capillaries, and *V* veins. *Ma* is a manometer connected with an artery to show changes in arterial

pressure. *Mv* is a manometer connected with a vein to show changes in venous pressure. The glass, containing water, represents the auricle; the bulb of the syringe, the ventricle. The in-

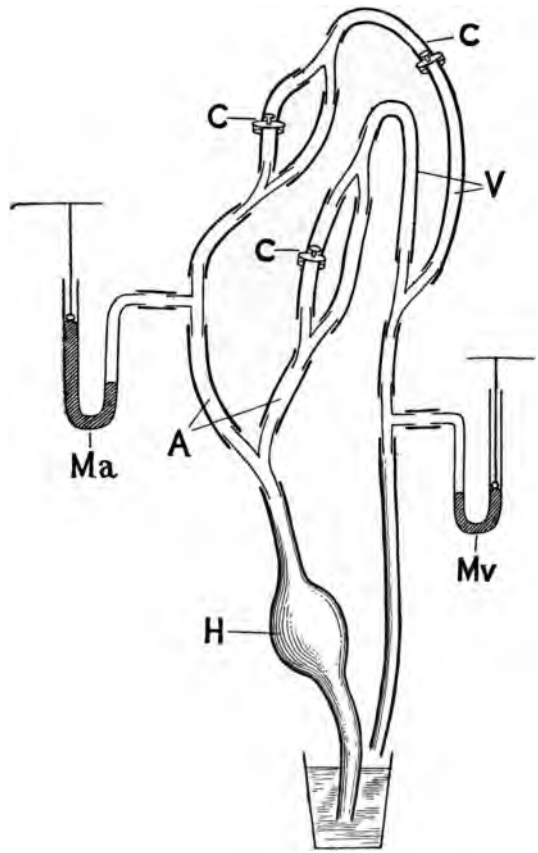

FIG. 33.—Artificial Schema of the Circulation. (Description in text.)

crease in resistance in the circulation that occurs in the capillary area is imitated by screw clamps on the tubing. The resistance may be increased by screwing the clamps more tightly, and de-

creased by loosening the clamps. Tightening the screws would imitate arterial constriction. Loosening them would correspond to a vaso-dilatation.

Before using the artificial schema, make out the following points. Connect the syringe with a long piece of glass tubing. When the syringe is filled, squeezing it with the hand will force fluid out through the glass tube. Release of the bulb will cause it to fill from the vessel in which the inlet tube is dipped. Press the bulb of the filled syringe with the hand. Note that water is forced through the glass tube and out of its free end in a jet which ceases as soon as the pumping force behind stops. Squeeze the bulb a number of times in succession. Note that there is no flow from the tube between pumps. In vessels with inelastic walls all the force of the pump is exerted in moving the column of fluid forward and in overcoming friction. The friction is inversely proportional to the size of the tube. The smaller the tube, the greater the friction. The longer the tube, the greater the friction.

For the glass tube, substitute a long rubber tube of small calibre. Press the syringe bulb a number of times in rapid succession. The water will still spurt from the open end of the tubing with each stroke of the pump, but there will probably be some flow between strokes. This may be increased and the flow during the stroke of the pump decreased by partly clamping the tube near the free end. In other words, the peripheral resistance has been increased.

If the peripheral resistance is sufficiently increased the flow becomes continuous. With elastic tubes and a high resistance to overcome, part of the force of the pump is expended in distending the walls of the elastic vessel. When the distending force has ceased, the elastic walls rebound and force the stored-up fluid on. With each stroke of the pump, then, in a system of elastic vessels some of the energy becomes latent in the distended walls of the vessels, to be transformed into kinetic energy in the interval between pumps.

With the clamps of the circulation schema open, press the syringe bulb a number of times in slow succession. Note the charac-

ter of the flow from the venous end of the schema. Is it continuous, remittent, or intermittent? Note also the excursions of the column of mercury in each manometer. Is there any difference in pressure as indicated by the two manometers? Explain.

Increase the peripheral resistance by tightening one of the capillary clamps. Repeat the intermittent pressure on the syringe bulb. Has the character of the flow changed? Compare the two manometers. Is there any change in arterial pressure as compared with venous pressure? Is the fall in arterial pressure between the strokes of the pump large or small? Does the venous pressure still rise and fall with the heart-beat? Explain.

Increase the peripheral resistance still more by tightening more clamps. Note the change in arterial pressure as compared with venous pressure, during the series of pump strokes. Note the change in the character of the outflow of fluid from the veins and the speed of flow as compared with the speed with lower resistance. Does the flow continue after the pump has ceased to act? Does the arterial pressure fall more slowly or more rapidly than before?

Is the excursion of the column of mercury in the arterial manometer greater or less with each pump stroke than before?

Decrease the rate of pump strokes. What is the effect on blood flow and on arterial pressure?

Dilate the arterioles by loosening a clamp. Using the same rate of pump strokes as before and as nearly as possible the same strength of stroke, what is the effect on arterial pressure and on venous flow?

Record of Pulse in Artificial Schema.—Close the clamps on the tubes representing the capillary circulation until, with freqeunt regular compressions of the pump, a constant outflow from the veins is obtained and the oscillations of the arterial manometer are slight. Determine the mean arterial pressure by multiplying the height of the column of mercury in the distal limb of the manometer above the meniscus in the proximal limb by 2.

Clamp off the manometer from the artery tube. On the artery near the bulb adjust a receiving tambour. Connect this with a re-

cording tambour, whose writing lever is applied to the smoked surface of a medium-slow drum. The increase in pressure at each stroke of the pump is partly employed in distending the vessel. This causes a bulging of the vessel and a hardening which can be felt by the finger and which in this case is transmitted through the receiving tambour to the recording tambour and is written as a curve on the revolving drum. This is the pressure pulse and is indicative of the rise in pressure in the artery with each systole of the ventricle. The lever of the recording tambour should be delicately adjusted to the surface of the drum so as to reduce friction to a minimum.

What is the form of the pressure pulse in this instance? How does the systolic rise compare with the diastolic fall? Are there any secondary waves? If so, what is their significance?

Increase the rate of the pump stroke. What change occurs in the pulse wave? Decrease the rate of the pump stroke. What change occurs in the form of the pulse wave?

Increase the peripheral resistance by tightening the clamps on the capillaries. What is the effect on the pulse? Decrease the peripheral resistance. What is the effect on the pulse?

With the capillary clamps so applied that the venous outflow is continuous, place the finger upon the venous tube while the heart bulb is rhythmically pressed. Is any pulse felt in the veins? Apply the receiving tambour to the vein. Is any pulse recorded by the recording tambour?

Release the compression in the capillary region until the venous outflow becomes remittent. Is a pulse tracing now obtainable from the veins? Explain.

XX. Pulse Record in Man.

By the Tambour Method.—A simple method for recording the pulse is by some such scheme as depicted in Fig. 34. This consists of a thistle tube, *T*, to act as a receiving tambour, and a recording tambour, *R*, connected with the receiving tambour by strong-pressure tubing for transmitting the impulse from one tam-

bour to the other. *R* is covered with a thin rubber membrane, drawn not too tightly, against which rests a long light lever. If the carotid pulse is taken, it is not necessary to cover the thistle tube with rubber. The integument over the artery, against which the tube is tightly pressed, acts as such.

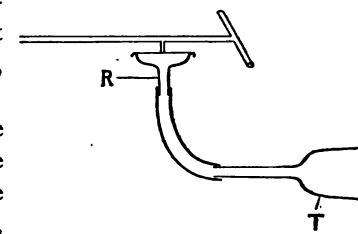

FIG. 34.—Simple Sphygmograph and Cardiograph. (Description in text.)

If a record of the radial pulse is to be taken, the thistle tube should be covered in the same way as the recording tambour, and a cork button cemented to the middle of the rubber membrane. The button is applied to the integument over the artery, the movements of the artery are transmitted to the button of the receiving tambour and its rubber membrane, and this in turn is transmitted to the membrane and lever of the recording tambour which writes the record upon the smoked paper of the revolving drum.

Take a tracing of the carotid pulse, adjusting the receiving tambour until a definite curve is obtained. Make out the general rise and fall of the pulse pressure with each systole and diastole and the secondary waves of the tracing. Explain.

Repeat, taking a record from the radial artery. Compare the sphygmographic record with palpation of the artery by the finger.

Take sphygmographic records with the spring sphygmographs of Marey or Dudgeon or some other similar instrument. Compare with the record taken by the tambour scheme.

XXI. VOLUME PULSE.

The pulse record as taken by the sphygmograph measures with a fair degree of approximation the changes in pressure in an artery brought about by ventricular systole and diastole, but measures very inexactly the changes in volume.

For this determination a larger vascular area is required. An instrument used for determining volume changes is called a *plethysmograph*. One of the simplest devices is that of Porter.

This consists of a glass tube, with a rubber collar made to fit snugly around the middle finger. The tube is connected with a recording tambour, and the volume changes of the finger are recorded upon a slowly revolving drum.

These changes are rhythmical in character and correspond to the rhythm of the heart-beat. In addition, larger waves may be written as a result of general or local vasomotor changes.

A larger record is obtained through the use of Mosso's water plethysmograph, by which the volume changes of the forearm are recorded.

This consists of a large glass cylinder provided with four openings—one for the insertion of the forearm, one for connection with the recording apparatus, one for filling the system with water, and one for the insertion of a thermometer.

A simple recording device is the water pen of Kronecker. This is a small box connected by rubber tubing with the plethysmograph cylinder upon the surface of the water in which a cork sheet, supporting a writing lever, is floated.

The hand and forearm are anointed with vaseline, a rubber collar is fitted snugly just below the elbow, the hand and forearm are inserted in the cylinder, and the rubber collar fitted about the flange of the opening through which the arm is inserted. The cylinder is connected with the recording apparatus, the cylinder and recording apparatus are filled with water, the thermometer is inserted in the opening provided for it, the filling bottle is clamped off, and the recording pen is applied to the surface of a slowly revolving drum.

Set up the apparatus as described above and take a normal-volume pulse tracing.

While the tracing is being recorded, elevate the free hand and arm above the head. Is there any change in volume of the arm in the cylinder?

Make a series of rapid shallow respirations. Is there any change in the tracing?

Make a series of deep slow respirations. What change occurs in the tracing?

Hold the breath for fifteen seconds. Note any change in the volume tracing.

While the tracing is being taken, work out some mathematical problem. What is the effect on the volume of the arm?

Immerse the free hand in ice water. This will cause a primary vaso-constriction in the vessels of the hand. Is there a corresponding vaso-constriction in the other hand, as indicated by a fall in volume?

Allow the subject of the experiment to take a few whiffs of amyl nitrite. This is a general vaso-dilatant. What is the effect on the plethysmographic tracing?

XXII. Apex Beat, Cardiogram, and Heart Sounds.

1. Let a student strip to the waist. Locate the apex impulse. What is its relation to the sternum? to the nipple? to the ribs and intercostal spaces? How does its position vary with the position of the subject?

2. Map out the cardiac area on the chest wall—(*a*) with light percussion; (*b*) with heavy percussion. Mark the area on the chest with a colored pencil.

3. Prepare a cardiograph as follows: Take a large thistle tube, about five centimetres in diameter, stretch a rubber membrane across the top and cement a cork button to the middle of the membrane. Connect this, through a piece of pressure rubber tubing, with a recording tambour whose diameter should be somewhat less than that of the thistle tube. In order to obtain sufficient magnification, the recording lever should be about twenty-five centimetres long. A good writing point may be cut out of a piece of the glazed paper used for tracings.

Adjust the writing point of the recording lever to the smoked paper of a medium-slow drum. Press the button of the receiving

tambour to the apex impulse. A curve will be written on the drum. Make a number of tracings from the same individual, varying the pressure of the cardiograph against the chest wall and varying the position of the subject of the experiment.

Compare the different tracings taken from the same individual with each other. Does the curve recorded consist of a single wave or of a wave with crests and depressions? Note the notches and apices of the waves which occur in all the tracings and the points in the curve in which they occur.

Take a series of tracings from another individual and compare the essential features of these with those of the other series.

With a stethoscope listen over the cardiac area for the heart sounds. Palpate the pulse at the wrist or over the carotid at the same time. How many different sounds do you hear? What are their characteristics in pitch, duration, and synchronism with the pulse wave?

At what part of the cardiac area is the first heart sound best heard? Where is the second sound most distinctly heard? What factors enter into the production of the heart sounds? Where can the mitral-valve factor be best made out to differentiate it from the other valves? The tricuspid-valve factor? The aortic-semilunar-valve factor? The pulmonary-semilunar-valve factor? Explain why you listen in certain regions for the different valve sounds.

While a cardiogram is being made, listen to the heart sounds. Compare the sounds, in point of time, with the features of the cardiogram tracing.

While a sphygmogram is being taken listen to the heart sounds.

XXIII. The Vasomotor Mechanism.

1. The General Controlling Centre in the Medulla.—Anæsthetize a large frog lightly with ether. Cut both vagus nerves, in order to exclude changes in the heart-beat, through inhibition or augmentation, from the result. Carefully avoiding hemorrhage, expose the brain and upper part of the cord.

A good index of general vasomotor changes is the speed of blood-flow through the capillary vessels of the frog's web. If the arterioles supplying the capillaries are constricted, the speed of flow through the capillaries will be diminished. If the arterioles are dilated, the speed of flow through the capillaries will be increased.

Keeping the brain and cord moistened with physiological salt solution, arrange the web under a microscope for observation with a medium high power. Note the size of the larger vessels, the arterioles and venules, and the speed of flow through the capillaries. The diameter of one of the arterioles may be measured by means of the micrometer eyepiece.

Excise the cerebral hemispheres and optic lobes, controlling hemorrhage, if necessary, by packing. Allow five or ten minutes for the frog to recover from the shock of the operation. Now examine the web again. Is there any observable change in the size of the arterioles or in the speed of blood-flow through the capillaries? Has there been any marked interference with the tone of the blood-vessels? What conclusion can you draw concerning the presence of a vasomotor centre in the parts excised?

Observe the web for some time. Are there any rhythmical changes in the diameters of the larger vessels discernible?

2. Inject a drop of saturated curare solution under the skin of the frog in order just to paralyze the motor nerves without affecting the innervation of the blood-vessels. This is done to keep the frog quiet during the ensuing stimulations. Set up an inductorium arranged for weak tetanizing currents.

While observing the flow through the web, stimulate the medulla with fine needle electrodes. What is the effect on the blood-flow through the web? Is this a constrictor or dilator effect?

3. Sever the medulla from the cord by a clean transverse cut with a sharp scalpel. Again observe the flow through the web. Is the speed increased or decreased? Is there a constriction or dilatation of the arterioles? What is the function of the centre in the medulla? Is its action constant or intermittent?

4. While observing the capillary flow, stimulate the cut end of the cord with a weak tetanizing current. What is the effect on the calibre of the blood-vessels? What conclusion can you draw concerning the function of the cord as a conductor of vasomotor impulses?

5. Cord Centres.—Allow the frog to rest for ten or fifteen minutes, making an observation of the web at frequent intervals. Is there any diminution in speed of flow? Is there any resumption of vasomotor tone? If so, what conclusion can you draw concerning the cord as a vasomotor centre?

6. Destruction of the Cord.—Expose the heart and vessels of the mesentery. Note the complete filling of the heart at each diastole and the size of the mesenteric vessels. Now insert a seeker into the spinal canal, destroying the cord. What is the effect on the filling of the heart and the abdominal vessels? What disturbance of the vasomotor mechanism has occurred through the destruction of the cord?

Examine the web. How does it compare with the condition which prevailed before the destruction of the cord?

7. Vasomotor Centres Outside of the Cord.—Pith another frog, but do not destroy the cord. Wait five minutes for the vasomotor tone to be re-established. Examine the web and note the size of the vessels and speed of flow.

Destroy the cord in the same way as before and note the change in the web flow. Observe the web at short intervals, for fifteen to twenty minutes, or longer if necessary. Is there any return of vascular tonus? Explain.

8. Vasomotor Reflexes.—Lightly curarize a frog. Carefully isolate the sciatic nerve of one thigh. Examine the blood-flow through the web of the opposite foot. Cut the nerve. Is there any effect on the capillary flow through the web? Is this effect constrictor or dilator?

After five minutes, stimulate the central end of the cut nerve with a weak tetanizing current. What is the result on the circulation through the web?

Examine the web of the foot on the same side as the cut nerve. Stimulate the distal end of the cut nerve. What is the effect on the capillary circulation? Explain.

Prepare another frog, cocainizing the sciatic nerve before cutting. Compare the web circulation in this case, before and after cutting, with the result obtained when the nerve was not cocainized.

XXIV. Record of the Blood Pressure in the Rabbit.

1. Preparation.—Under the skin of the rabbit inject ½ grain of morphine sulphate. Anæsthetize lightly with ether. Expose both carotid arteries, and isolate and identify the nerves running with them. Pass two loops of thread about the vagus and depressor nerves of each side. Introduce a cannula into the trachea. Clean the carotid of one side for a distance of three or four centimetres. Pass two loops of thread around the artery. Tie the upper ligature as far away from the heart as possible. Clamp the artery near the lower part of its isolation. With a pair of fine-pointed scissors make a V-shaped opening in the vessel near the upper ligature, using the tied ligature to manipulate the artery. Introduce a fine blunt-pointed seeker into the lumen of the cut vessel toward the heart. Using this seeker as a guide, insert into the artery a glass cannula made for the purpose. Tie this securely in the vessel by means of the remaining untied ligature.

In the mean time the apparatus for transmission and recording of the blood pressure should have been made ready. This consists of a revolving drum, covered with smoked glazed paper; a mercury manometer with an upright and writing style resting on the mercury of one limb of the manometer and arranged to write on the drum; the other limb of the manometer is connected through pressure tubing with the cannula which has been introduced into the carotid.

The proximal limb of the manometer, and the tubing connected with it, are filled from a pressure bottle, with a half-saturated solution of sodium carbonate or some other salt solution to prevent clotting of the blood which may find its way into the tubing.

The manometer tubing is clamped off and the pressure in the manometer and connections is raised to approximate the estimated arterial pressure of the rabbit.

The artery cannula is then filled with 0.8-per-cent solution of NaCl, care being taken to get rid of all air bubbles both in the cannula and manometer connections. The manometer tube is then connected with the arterial cannula, the clamp is removed from the tubing and from the artery at the same time, and the pressure in the artery is transmitted through the tubing to the mercury in the proximal limb of the manometer which falls and rises in the distal limb.

Before the mercury in the manometer was put under extra pressure, a base line should have been drawn around the drum to indicate the atmospheric pressure. The height of the tracing above this base line, multiplied by 2, gives the pressure in terms of mercury.

2. Note the rise and fall of pressure synchronous with the heartbeat. Where the beat is rapid, as it is normally in the rabbit, the excursion of the manometer style is not an accurate index of the variations of pressure. The inertia of the mercury is too great to follow exactly small and rapid variations in pressure.

Note the larger waves in the tracing. These are due to changes in arterial pressure brought about by inspiration and expiration. Observe the respiratory movements and compare them with the respiratory waves of the tracing. Is there a rise or fall of blood pressure with inspiration? with expiration? Does the change correspond exactly to the respiratory movement?

Are there any other pressure waves in the tracing aside from the pulse waves and the respiratory waves? Explain.

3. Effect of Vagus Stimulation.—While the tracing is being taken, tie one vagus with two ligatures and cut between. Note any change in the pressure tracing. Mark on the tracing the place at which the nerve was tied and cut.

(*a*) Stimulate the peripheral end of the cut nerve with a weak tetanizing current. Is there any effect on blood pressure or upon

the rate and strength of the heart-beat? Stimulate, again, with a somewhat stronger current and note the effect.

Increase the strength of the current, a little each time, until the full inhibitory vagus effect is obtained. What is the result on blood pressure? What is the after-effect of vagus stimulation?

(*b*) Stimulate the central end of the divided nerve with medium strong tetanizing current. What is the effect on blood pressure? Note the respiratory movements before and during the stimulation of the central end of the nerve.

4. Depressor Stimulation.—Tie the two ligatures which were passed around this nerve and cut the nerve between the ligatures.

(*a*) Stimulate the peripheral end of the cut nerve (the end toward the heart) with medium strong tetanizing currents. Is there any effect on the rate or strength of the heart-beat or upon the blood pressure?

(*b*) Stimulate the central end of the divided nerve in the same way. Is there any effect on the rate and strength of the heart-beat or upon the blood pressure, or both? How does this tracing compare with that obtained through stimulation of the peripheral end of the cut vagus, using the same strength of stimulus?

Compare the result obtained through stimulating the central end of the depressor with that obtained through stimulating the peripheral end. Is the depressor effect a direct or an indirect effect? Is the nerve an efferent or an afferent pathway? If it forms part of a reflex arc, what nerve pathways form the other limb or limbs of the arc?

(*c*) Now cut the intact vagus of the opposite side. Note any further change in the blood-pressure tracing.

Again stimulate the central end of the depressor nerve. Is there any fall in blood pressure? Is there any slowing of the heart-beat? What change has vagus section made in the depressor reflexes? Explain. In the light of the above observations, what are the functions of the depressor nerve?

5. Section of the Cervical Sympathetic.—Using the same rabbit, observe the appearance of the blood-vessels in the two ears.

Secure the cervical sympathetic nerve on one side with two ligatures. Cut the nerve between the ligatures. After a short time, again compare the two ears. Is there any change in vascularity of the ear of the side upon which the nerve was cut as compared with the ear of the sound side?

While observing the two ears, stimulate the peripheral end of the cut nerve. What change occurs in the blood supply to the ear of the stimulated side? Note any other phenomena which occur during stimulation of the cervical sympathetic nerve. What is the function of the nerve as far as the vascular supply to the ear is concerned? How does the temperature of the ear on the operated side compare with that of the ear on the sound side?

Locate the cardiac impulse on the chest wall of the rabbit and introduce into the heart a fine knitting-needle. This will move with the heart-beat and serve as an indicator. Place a bit of absorbent cotton saturated with chloroform over the end of the tracheal cannula. Note the effect on the heart-beat. Kill the animal with chloroform. Note which stops first, heart-beat or respiration. After the respirations and excursions of the heart needle have both stopped, open the thorax and observe whether or not the heart is still feebly beating.

6. Effect of Hemorrhage on Blood Pressure.—Prepare a rabbit, as before, for a blood-pressure record. In addition introduce a long glass cannula into the opposite carotid artery and prepare the jugular vein for transfusion.

Take a normal blood-pressure record for comparison. While this is being taken, remove the clamp from the opposite carotid until 50 c.c. of blood have been shed. Note any change in blood pressure during the bleeding. Shut off the artery after the loss of the 50 c.c. of blood. If the pressure has fallen during the hemorrhage, does it continue low or does it recover after the bleeding has ceased? Explain.

Bleed again another 50 c.c. of blood. Is there any further fall in pressure? If so, does it again rise after the cessation of the hemorrhage?

Bleed again, if necessary, until the blood pressure ceases to recover. Now introduce into the jugular vein, slowly, 0.8-per-cent NaCl solution, until 50 or 100 c.c. have been transfused. Does the pressure again rise? If not, continue the perfusion.

Perfuse four or five times as much of the salt solution as there was blood lost. Is there any noticeable rise of pressure above the normal? If not, explain

7. Shock and Blood Pressure.—Prepare another rabbit for blood-pressure recording, isolating the vagus and depressor nerves at the same time. Take a normal blood-pressure tracing. While this is being done, stimulate the peripheral end of the vagus. Take another tracing, stimulating the depressor nerve. These tracings will serve as checks to the results obtained later.

While the pressure tracing is being taken, open the abdomen by a long median incision. Expose and handle the abdominal viscera. What is the effect on the blood pressure?

If the pressure has fallen, stimulate the depressor nerve. Does the pressure fall still more?

While the pressure is low, inject into the jugular vein 2 c.c. of a 1 to 10,000 solution of adrenalin chlorid. What is the effect on blood pressure and rate of the heart-beat?

The adrenalin effect will disappear after a short time. Cut both vagus nerves and repeat the adrenalin injection. Is there any difference in effect on the rate of the heart-beat as compared with the first result obtained?

XXV. Perfusion of the Rabbit's Heart.

Bleed the rabbit of experiment 7 from both carotids, and defibrinate the blood as shed. Mix this with equal parts of 0.8-per-cent NaCl solution which has been warmed to 37° C.

Open the thorax. Excise the heart which will probably continue to beat. Introduce and tie a small cannula into the opening of one coronary artery where it leaves the aorta. Free the cannula of air by filling it with physiological saline. Place the defibrinated blood mixture in a bottle with a side piece and resting in a water-

bath kept at a constant temperature of 38° C. Raise this perfusion bottle above the heart to give a pressure of one hundred millimetres of mercury.

Connect the perfusion bottle with coronary cannula, place the heart in a normal salt-solution bath, and begin perfusion. Note the strength and rhythm of the heart-beat as well as the rate. Is the mammalian heart dependent upon the central nervous system for the origination of its beat or for the co-ordination of the beat?

After twenty minutes, if the heart is still beating strongly, disconnect from the defibrinated-blood perfusion, and perfuse, instead, with warm 0.8-per-cent NaCl solution. How does this solution compare with the blood mixture in maintaining the beat of the heart?

XXVI. Effect of Temperature on the Heart-beat.

Narcotize and etherize a medium-sized rabbit. Place on rabbit-board, back down. Expose both carotid arteries, in the neck region. Pass under both arteries a large glass tube connected with a pressure bottle. Insert in the heart, through the thorax, a knitting-needle indicator. Count the heart-beats per minute. Also count the respirations. Now allow water, heated to 40° C., to flow through the tube running under the arteries. Note the increase in the rate of the heart-beat and in the frequency of respiration.

Stop the flow of the hot water through the tube. Allow the heart-beat and respiratory rate to return to normal and then run ice water through the tube. What is the effect of the cold as compared with the application of heat to the circulating blood?

In all of the experiments on the rabbit where it has not been specified, the animal should be narcotized with morphine and anæsthetized with ether.

XXVII. Estimation of Human Blood Pressure.

A number of instruments have been devised for estimating blood pressure in man. It is obviously impracticable to determine the

blood pressure in the same way that was done in the case of the rabbit.

The Riva-Rocci sphygmomanometer or some modification of it is in most general use, and, although not the most accurate form of apparatus, perhaps, for the purpose, it certainly is most convenient.

The apparatus consists of an elastic tube, blind at both ends and having a side piece for connection with the air-inflating pump (see Fig. 35). This tube is covered by a leather cuff, so that when it is

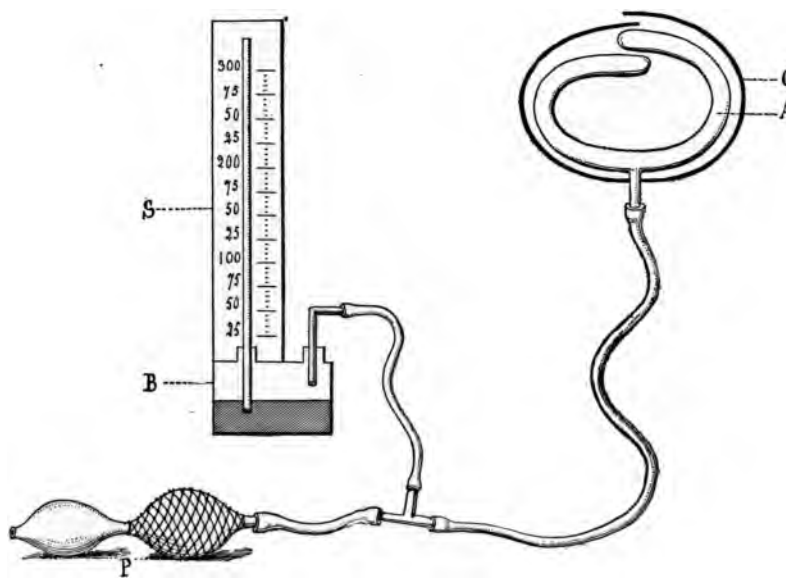

FIG. 35.—Sphygmomanometer (Schematic). (Description in text.)

adjusted around the arm or forearm the pressure is exerted in the direction of the limb (see Fig. 35, *A* and *C*). The arm tube is connected with some form of air pump (Fig. 35, *P*) and with a mercury manometer (Fig. 35, *B*). By inflating the tube until sufficient pressure upon the arm is produced to shut off the pulse at the wrist, a rough estimate of the systolic blood pressure is obtained. This

pressure is read off on the manometer scale in millimetres of mercury.

If now the pressure is released until the widest oscillations of the mercury meniscus are just secured and a reading is then taken, the diastolic pressure is shown. This may be more accurately determined by palpating the pulse and inflating the tube until the first perceptible diminution of the pulse occurs. To reduce error as much as possible, the arm tube and cuff should be at least twelve centimetres in width.

With some such instrument as that described above, make a number of determinations of both systolic and diastolic pressure of one individual. Repeat the observations on other individuals of the class. If there are other instruments at hand for this same purpose, make observations with several different instruments on the same individual.

Make an observation on a normal individual. Allow the subject to take several whiffs of amyl nitrite and observe the blood pressure.

CHAPTER VI.

SECRETION—DIGESTION—ABSORPTION.

I. Secretion of Saliva.

Mechanism of Secretion.—*Demonstration.*—Secretion may be divided into two stages—the productive stage, during which the secretory products are being formed in the gland cells, and the eliminative stage, during which the products already formed pass out of the cells and through the gland ducts to the surface where the secretion performs its particular function.

The elimination of the secretion may be brought about either by a nervous stimulus of the gland cells or a chemical stimulus, or both. The mechanism of the elimination of the salivary secretions furnishes an excellent example of the influence of nervous impulses. This is not of so much importance in itself, as it is in serving as a type of the nervous mechanism of secretions in general.

Of the salivary glands, the one which has been chiefly studied and used to demonstrate this mechanism is the submaxillary gland.

Place a dog under ether and tie to the dog-board. Clip the hair from the jaws and neck and shave the skin. Make an incision through the skin of the lower jaw, along its inner border, beginning just in front of the insertion of the anterior belly of the digastric muscle and extending backward through the platysma muscle to the transverse process of the first cervical vertebra.

Expose the jugular vein and its branches, including those which drain the submaxillary gland. Tie all venous branches which pass below and in front of the gland, excepting those which come from the gland itself. The veins should be tied between two ligatures,

and the intervening portion excised. Clean the masseter and digastric muscles of cellular tissue. Avoid injury to the facial artery and the gland duct which lies between it and the masseter muscle. Carefully separate the digastric muscle from the artery, and tie the branch which supplies the muscle. Divide the digastric and mylo-hyoid muscles and, being careful to avoid injury to the structures beneath, turn the muscle flaps back.

The submaxillary gland should also be gently drawn upward and backward. The following structures will now be exposed to view:

In front of the posterior insertion of the digastric and in front of and below the reflected gland, the carotid artery is seen. Crossing this is the hypoglossal nerve, *H* (see Fig. 36), and running along

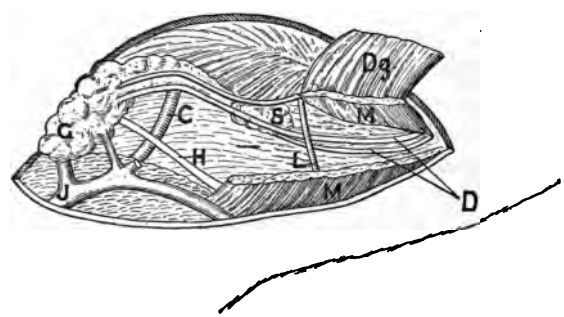

FIG. 36.—Dissection of Submaxillary Region, Dog. (After Bernard.) *G*, Submaxillary gland; *J*, jugular vein; *C*, carotid artery; *H*, hypoglossal nerve; *L*, lingual nerve; *T*, chorda tympani; *D*, ducts of submaxillary and sublingual glands (*S*); *Dg*, digastric muscle; *M*, mylo-hyoid muscle.

with the artery are filaments of the sympathetic nerves. Entering into and passing out of the hilum of the gland are seen the chorda tympani nerve branch to the gland, the branch of the facial artery to the gland, and the gland duct.

Beneath the reflected mylo-hyoid muscle is seen the lingual nerve (Fig. 36, *L*). Trace this nerve to the ramus of the jaw. At this point a small branch will be exposed which, in close proximity

to the duct, runs backward to the gland. This is the chorda tympani (Fig. 36, *T*).

Pass a thread under the chorda to use later in handling the nerve for stimulation. Divide the hypoglossal nerve and expose the sympathetic filaments. Pass a thread around these also. Identify the submaxillary duct and introduce and tie a cannula into it. The cannula should end in a small rubber tube. This is closed by an artery clip until it is desired to collect the secretion. The introduction of the cannula may be facilitated by first stimulating the chorda for a short time with a weak tetanizing current, thus distending the duct with secretion.

Small graduated glass cylinders are provided for collecting the secretion from the cannula in the gland duct. These may be changed at any desired interval of time, say every five or ten minutes. The rate of flow is determined by the amount of secretion eliminated in a given time period.

1. Observe the rate of flow from the gland before stimulation. Has the anæsthetic any stimulating influence on the salivary flow? This observation should cover a period of five minutes.

2. Stimulate the chorda with a weak tetanizing current. How is the rate of salivary flow affected? Compare the appearance of the blood-vessels of the gland during stimulation of the nerve with the vascular condition before stimulation.

3. Allow the preparation to rest for several minutes. Stimulate the sympathetic. Is there any marked effect on the rate of flow? What is the effect upon the condition of the blood supply to the gland?

4. Paint the submaxillary ganglion with a 0.1-per-cent solution of nicotine. Nicotine, in weak solution, paralyzes nerve cells, but not nerve fibres. Stimulate the chorda again. Is there still an accelerator effect on the salivary secretion? Are the fibres of the chorda broken by nerve cells in this ganglion?

Now paint the chorda with nicotine where it enters the hilum of the gland. Stimulate the chorda again. Is there any effect on the flow of the secretion? Stimulate at the hilum itself. Is there any

effect on the flow of secretion? Does the chorda run directly to the gland cells without interruption? If not, where is the point of transfer?

5. Wash the gland thoroughly with warm physiological saline. After a time the nicotine effect will wear off. Isolate and introduce a cannula into the femoral vein, centrally. Inject into the vein ¼ grain atropine sulphate. Note the effect on the flow of saliva. Stimulate the chorda. Is there an increased flow of the secretion? Empty the duct of secretion. With a hypodermic syringe inject into the duct a few drops of a 2-per-cent solution of pilocarpine nitrate. Stimulate the chorda again. Is any effect now obtainable on the salivary flow? After several minutes stimulate again. The atropine effect has once more asserted itself.

Does stimulation of the nerve still cause a dilatation of the gland vessels? Is the effect of the nerve stimulation in causing an increase in flow of secretion purely a vaso-dilator effect or is it due to a stimulation of the gland cells themselves? Explain.

II. To Show Changes in the Gland Cells Following Chorda Stimulation.

Make another preparation of submaxillary gland, duct, and nerve. Stimulate the nerve with a weak tetanizing current until the flow of the secretion ceases. Allow an interval of five minutes' rest and repeat the stimulation. Continue to complete exhaustion of the gland. Remove both submaxillary glands. Cut out small portions of each and make frozen sections of the fresh glands. Mount in normal salt solution or in glycerin. Examine sections from the two glands under the microscope. Compare the appearance of the cells of the stimulated gland with that of the resting gland.

Harden the remainder of the two glands in absolute alcohol; embed in paraffin; cut sections; and stain with carmine. Compare the stained sections of the two glands with each other and with the frozen sections of the fresh glands.

III. Salivary Digestion.

1. Chemical Constituents of Saliva.—Chew a piece of paraffin gum or inhale a little ether vapor. The flow of saliva is thus stimulated. Collect the secretion in a clean porcelain capsule. Filter and divide the filtrate into five portions.

(*a*) Test the first portion for its reaction with litmus paper. Is it alkaline or acid? Is the reaction very decided in either direction?

(*b*) To the second portion add dilute acetic acid. The presence of mucin is indicated by the formation of a precipitate.

(*c*) To a third portion add a few drops of a silver-nitrate solution. A precipitate of silver chlorid which is soluble in ammonia and insoluble in nitric acid is indicative of the presence of chlorids.

(*d*) To another portion add dilute acetic acid and filter. Test the filtrate with Millon's reagent. The presence of proteids is shown by the production of a red coloration or precipitate.

2. Action on Starches.—(*a*) To some boiled starch paste add a few drops of iodine. A blue coloration will occur. To some powdered starch add a few drops of iodine. A blue color test will also be obtained. Both cooked and raw starch respond to the iodine test.

(*b*) To a test-tube partly filled with a dilute Fehling's solution add a little of the starch paste. Boil the mixture. There should be no reduction of the copper sulphate of the Fehling's solution. The copper salt is reduced by any of the reducing sugars.

(*c*) To another portion of Fehling's solution add a few drops of a dilute solution of dextrose. Heat to boiling and note the formation of a copious precipitate, first yellow, and, as the heating is continued, changing to a reddish color. This is the cuprous and later cupric oxide formed by the reduction of the copper salt in the test solution.

(*d*) Repeat the test with maltose instead of dextrose. Is reduction obtained?

(*e*) To another portion of the test solution add lactose. Is the copper salt reduced?

(*f*) Test a solution of cane sugar for reduction. Result?

(*g*) Partly fill three test-tubes with starch paste. Mark them I, II, and III. To I add some of the filtered saliva. To II add some saliva that has been heated to boiling. To III add some saliva that has been neutralized with HCl and has had enough acid added to bring the acidity up to 1 per cent HCl. Place the three tubes in a water-bath or in the incubator kept at a temperature of 38° C. In five minutes remove the tubes and test the three for reducing sugar with Fehling's solution. Is there any reducing sugar in I? in II? in III? Explain.

(*h*) Make a solution of dextrin. To this solution add a few drops of iodine. Note the wine-color reaction.

(*i*) To a few cubic centimetres of the dextrin solution add an equal quantity of saliva. Place in the water-bath or incubator for one hour at a temperature of 38° C. At the end of this time test the solution with iodine for the red dextrin reaction. Is there any color reaction? Test for reducing sugar.

(*j*) Mix equal quantities of boiled starch and saliva in a test-tube. Place in the warm water-bath. Place several drops of dilute iodine solution on a white porcelain slab. At five-minute intervals, by means of a glass stirring-rod, add a drop of the digesting starch to a drop of the iodine on the slab. Continue this until a color reaction is no longer obtained. What changes occur in the color reaction as digestion progresses?

(*k*) Take some boiled starch into the mouth and go through the movements of mastication. At the end of one minute spit this out into some boiling water in a beaker. Test a portion of the mixture for sugar, and another portion for starch. Repeat with another portion of starch, keeping it in the mouth two minutes. Chew another portion for five minutes and test again for starch and sugar. Does the test for starch diminish in intensity, and the test for sugar increase?

(*l*) Mix a portion of fibrin in a test-tube with some saliva.

Place in the incubator for twenty minutes. At the end of this time remove and test the mixture for peptones. Has any of the proteid been digested?

(*m*) Add some saliva to a starch solution in a dialyzer tube of parchment paper for twenty-four hours in the incubator. At the end of this time test the water surrounding the dialyzer for reducing sugar and for starch.

IV. Mechanism of Swallowing.

1. Inject a solution of 0.03 gram morphine sulphate under the skin of a medium-sized rabbit. Anæsthetize lightly with ether. Tie the rabbit, back down, upon the rabbit-board, with the neck well stretched out. Clip and shave the hair in the neck region and also over the epigastrium and zyphoid appendix.

2. Make a median incision through the skin and fascia of the neck. Separate the sterno-hyoid muscles and expose the trachea. Carefully separate the trachea from the œsophagus, which lies behind it, avoiding injury to the nerves and vessels running beside and between the two.

3. On either side of the trachea and between it and the œsophagus, a fine nerve filament will be seen. These are the recurrent laryngeal nerves. Pass a loop of thread around each recurrent laryngeal nerve, but do not tie. Isolate the vagus nerve of each side and secure with untied threads. Follow the vagus on one side as far as the level of the lower part of the thyroid cartilage. At this point it is joined by the superior laryngeal nerve. Isolate and pass a loop of thread around this nerve.

4. Pass a heavy ligature around the trachea as low in the neck as practicable. Cut between the rings of the trachea just above this ligature. Introduce a tracheal cannula and tie it in with the ligature. Excise the piece of trachea between the cannula and the cricoid cartilage. This brings the œsophagus well into view. Pass two threads around the œsophagus, to be tied later.

5. Now make a median incision through the abdominal wall in the median line and expose the stomach. By pulling the stomach

down with one hand, and the liver and lower ribs up with the other, the junction of the œsophagus and stomach may be seen.

6. Arrange an inductorium for weak tetanizing current. Place the electrodes from the secondary coil under the superior laryngeal nerve. Arrange a metronome or chronograph marking seconds. This is to assist in taking the time of the swallowing movements.

The work of the experiment may be divided among four students as follows:

Let one student manage the stimulation of the superior laryngeal nerve or other nerves that it may be desired to stimulate during the course of the experiment; let another make the time observations of the swallowing movements; let a third manipulate the stomach for observation of the lower end of the œsophagus; and let a fourth make careful notes of the observations.

7. Stimulate the superior laryngeal nerve with the weak tetanizing current until the rabbit swallows. Stimulation of this afferent nerve brings about, among other things, a reflex swallow. Note and time the beginning of the swallowing movement. Note the passage of the peristaltic wave along the cervical portion of the œsophagus, and the end of the peristaltic movement at the stomach. How much time has elapsed between the beginning of the swallow and the ending of the peristalsis at the stomach? Repeat the observation a number of times. What is the average time occupied by the passage of a peristaltic wave over the length of the œsophagus in your rabbit?

8. Determine whether the mechanism of œsophageal peristalsis is a nervous reflex one or due to muscular conduction of the contraction wave from one segment of the œsophagus to another.

Tie two ligatures around the œsophagus, in the cervical region, and cut completely through the gullet between the ligatures. Muscular continuity is thus absolutely severed.

While making observations, as before, produce a swallow by stimulating the superior laryngeal. Does the peristaltic wave still pass over the lower segment of the œsophagus to the stomach? If

so, how does the time occupied in the passage of the wave compare with the time before the gullet was divided?

If the peristaltic wave ceases to pass below the cut, what conclusion might you draw concerning the method of conduction of the contraction wave? If the wave continues to pass after the severance of muscular continuity, what conclusion might you draw?

9. Secure the vagus of one side with two ligatures and cut between. Again induce a swallow as before. Does the contraction wave still pass over the œsophagus? Explain.

10. Now cut the other vagus also. Both vagus nerves are now cut. Induce a swallow. Does the peristaltic wave continue to pass over the œsophagus?

From the above observations what conclusions can you draw concerning the mechanism of œsophageal peristalsis and the function of the vagi in this connection?

11. The Vagus as a Motor Nerve to the Stomach.—Enlarge the abdominal incision so as to expose the whole of the stomach including the beginning of the duodenum. Place the peripheral ends of both vagi upon electrodes from an inductorium arranged for medium strong tetanizing currents. Stimulate both vagi continuously and note the strong contraction rings which pass over the stomach from the fundus toward the pylorus. Note the opening of the pyloric sphincter and the expulsion of a small quantity of stomach content into the duodenum. Note the movements of the duodenum during and after the entrance of food from the stomach. Keep the stomach covered with a pad of absorbent cotton moistened with warm physiological saline, between observations.

Over what part of the stomach wall are the contraction rings most distinct and strongest? Where do they begin and in what directions do they pass?

12. Stimulate both inferior laryngeal nerves. Note the effect upon the upper segment of the œsophagus. What is the nature of the musculature of the first part of the œsophagus?

13. Free the small piece of trachea connected with the cricoid cartilage from blood and note the position of the vocal bands.

Note the slight opening and closing of the glottis with inspiration and expiration. While observing the movements of the vocal bands, stimulate one inferior laryngeal nerve. What is the effect on the vocal bands? Stimulate both nerves at the same time. What is the effect on the movements of the vocal bands?

14. Stimulate the superior laryngeal nerves in the same way and note the effect, if any, on the vocal bands.

15. *To determine the time occupied for the passage of a liquid from the mouth into the stomach, in man*, proceed as follows:

Arrange a drum with smoked paper for medium slow revolution. Set up a chronograph to mark seconds on the drum. Place a short-circuiting key in circuit with the time marker. When the key is closed, the lever of the time marker will write a straight line. When the key is opened, a time tracing, in seconds, will be recorded. Take a fellow-student into a quiet room and listen with a stethoscope over the end of the sternum. Start the drum. Let the subject of the experiment take one swallow of water. You will hear two sounds, one when the liquid is shot into the œsophagus, the other when the liquid enters the stomach. When the first sound is heard, open the short-circuiting key. When the second sound is heard, close the key. How many seconds have elapsed between the two sounds?

V. Gastric Digestion.

1. Tests for Proteids.—(*a*) *Coagulation by Heat.*—Prepare solutions of the following proteids: A, egg albumin, dilute; B, acid albumin in acid solution. This is obtained by subjecting some dilute egg albumin to the action of 0.2-per-cent HCl for several hours at body temperature. Neutralizing with an alkali will precipitate the acid albumin from its solution.

C, myosin, dissolved in a 10-per-cent NaCl solution. This may be prepared by mincing lean meat, freeing from blood by repeated washings, and extracting the myosin by an ammonium-chlorid solution. The salt may be removed by dialysis, leaving the myosin as a gelatinous mass, or it may be precipitated by diluting the solu-

tion with distilled water. This precipitate is redissolved in the sodium-chlorid solution as given above.

D, proteose. This may be prepared by digesting a small quantity of fibrin with 0.2-per-cent HCl and a little commercial pepsin, at 38° C., just to the point of solution of the fibrin and no more. Neutralize carefully with dilute NaOH, heat to boiling, and filter. Witte's peptone may be used, since it consists chiefly of albumoses.

E, peptone. Savory & Moore's preparation is used.

Place a small quantity of each of the above solutions in test-tubes and immerse in a water-bath heated to 65° C. Gradually raise the temperature of the bath to 100° C., noting observations at every 5° rise in temperature.

Are all of these solutions coagulated by heat? In those in which coagulation does occur, is the coagulating point the same?

(*b*) *Nitric-acid Ring Test.*—Place a small quantity of HNO_3 in a test-tube. With a glass tube of small calibre draw up some of solution A into the tube, and with the finger firmly pressed over the other end introduce the end containing the solution into the acid. Remove the finger. If the acid level is slightly higher than the level of the liquid in the tube, some of the acid will be drawn up into the glass tube containing the solution to be tested. Is there any ring of precipitation formed where the two liquids come in contact? Repeat this test for the other proteid solutions and record results.

(*c*) *Xanthoproteic Reaction.*—Add an excess of concentrated nitric acid to a little of each of the above tested solutions and heat to boiling. A yellow color is produced. Neutralize and make the solutions alkaline with sodium hydrate or ammonia. The color changes to an orange red.

(*d*) *Biuret Test.*—Make the solutions of the proteids to be tested alkaline with sodium hydrate. Add a few drops of a dilute cupric-sulphate solution. Be careful not to add an excess of the copper solution, since this may give a test in the absence of proteid. A blue-purple or violet color results.

(*e*) *Millon's Test.*—Test each of the solutions with Millon's

reagent, which is already made up. This is a solution of mercurous nitrate together with some free nitrous acid. When mixed with proteid a yellow precipitate is formed which becomes red on heating.

2. To Differentiate Albumins, Proteoses, and Peptones.—(*a*) What reactions have they in common? Are the albumins coagulable by heat? Are the proteoses coagulated by heat? Are the peptones coagulated by heat?

(*b*) Do the proteoses give a precipitate with nitric acid? Do the peptones?

(*c*) To a proteose solution add some potassium ferrocyanid acidified with acetic acid. Is there any precipitate? Repeat with a pure peptone solution. Is there any precipitate?

(*d*) To a solution of proteoses add sodium chlorid to saturation. Is there any precipitate? Repeat with pure peptone solution.

(*e*) To a peptone solution add alcohol. Is a precipitate formed? Repeat using a saturated solution of tannic acid instead of the alcohol. Result?

3. Artificial Gastric Juice.—Scrape off the mucous membrane of the fresh stomach of a pig. Grind this thoroughly with clean sand in a mortar. Add ten times the volume of a 0.2-per-cent solution of hydrochloric acid and place in the incubator at blood temperature for twenty-four hours.

Grind up another portion of mucous membrane of the pig's stomach with glycerin. Let this stand for several days before using.

(*a*) To a little fibrin in a test-tube add some 0.2-per-cent HCl.

(*b*) To another portion of fibrin add some of the glycerin extract of the pig's stomach.

(*c*) To another portion of fibrin add some of the acidulated aqueous extract of pig's stomach.

(*d*) To another portion add some of the acidulated extract which has been neutralized and made slightly alkaline with sodium carbonate.

(*e*) To another portion add some glycerin extract plus enough

0.2-per-cent HCl to make the acidity of the mixture equal to about 0.1 per cent HCl.

(*f*) To another portion add glycerin extract plus 0.4-per-cent acetic acid.

(*g*) To another portion add glycerin extract plus 0.4-per-cent lactic acid.

Make the fibrin portion in each tube as near the same quantity as possible and use the same amount of the extract each time. Place all the tubes, properly labelled, in a water-bath kept at a temperature of 38° C. Examine the specimens every three to five minutes, and note the extent of the solution of the fibrin in each tube. Continue the observations for a half-hour. Solution of the fibrin is not an index of complete digestion, but is a sufficient index for rough comparison of the digestive activity of the mixtures in the various tubes.

Note results and record observations. The glycerin extract contains pepsin, but no acid. What is the digestive activity of pepsin in the absence of acid? What is the digestivity of HCl alone, in the absence of pepsin? Is there any digestion with the alkaline mixture of pepsin? Does digestion occur when other acids are substituted for HCl?

(*h*) Boil some of the acidulated glycerin extract. Add this to fibrin and note results. Does heat destroy the enzyme?

4. Filter the contents of one or more of the tubes in which the fibrin has been dissolved.

(*a*) To a portion of this filtrate add dilute NaOH, carefully, until the acid is neutralized. Is there any precipitate? If so, the presence of what proteid is indicated? Filter.

(*b*) To a part of this filtrate add an excess of the alkali and then a drop or two of very dilute $CuSO_4$ solution. How is the presence of proteoses and peptones indicated? What is the necessity for care in adding the copper-salt solution?

(*c*) Heat another part of the filtrate from (*a*) to 70° C. Is there any coagulation?

(*d*) Take another portion of the filtrate from (*a*) and saturate

the solution with ammonium sulphate. How is the presence of proteoses indicated? How are they differentiated from peptones?

(*e*) Filter portion (*d*) and test the filtrate for the biuret reaction. If present, what does it indicate?

(*f*) Take a portion of the fibrin which has been acted upon by the acidulated glycerin extract, neutralize the mixture with sodium carbonate, and place in a dialyzer over night. The next day test the dialyzant for albumin, proteoses, and peptones.

Test the fluid remaining in the dialyzer in the same way. Results? Conclusions?

5. Place some fresh milk in a test-tube, in a water-bath at 38° C., and add a few drops of rennet extract. What is the action of the rennin? Remove the fluid part of the milk so far as possible, and add some artificial gastric juice. Is the casein dissolved?

VI. Intestinal Digestion.

1. Emulsification.—(*a*) Shake up 5 c.c. of olive oil in a test-tube with an equal quantity of water. The mixture will become milky white because of the distribution of oil globules through it. Do these oil globules remain in suspension?

(*b*) Repeat the procedure in (*a*), adding to the mixture, before shaking, 5 c.c. of strained egg albumin. Do the oil globules remain in suspension? Set this to one side and observe again, some hours later. Has any of the oil begun to separate out?

(*c*) Repeat (*b*), substituting gum acacia for the egg albumin. Set aside and observe later for the separation of oil from the emulsion.

2. Saponification.—To 5 c.c. of olive oil or cottonseed oil add twice the volume of a 20-per-cent solution of NaOH or KOH. Shake well. Continue to agitate the mixture at frequent intervals for fifteen to twenty minutes. Now add an excess of water. Has the oil been dissolved? What chemical reaction has taken place between the oil and the alkali?

3. Saponification as an Aid in Emulsification.—(*a*) Mix a small quantity of oil which has become slightly rancid, with a

strong solution of sodium carbonate. Shake the mixture vigorously. Set the emulsion thus formed aside and compare it later with the emulsions made with egg albumin and gum acacia.

(*b*) Repeat (*a*), but do not shake the mixture. Is an emulsion produced? Place some of this mixture under the microscope and observe the disintegration of the oil-drops into small globules. How does this compare with the emulsification of fats in the intestine?

4. A pancreatic extract may be made in the following way. Take a pig's pancreas or a dog's pancreas which has lain for twenty-four hours at the room temperature. Cut into small pieces or run through a meat grinder. Crush in a mortar with twice its volume of glycerin. Place the mixture in a bottle and allow to stand for several days before using.

For use, strain the mixture through a fine cloth and dilute as needed with four or five times its volume of water containing sodium carbonate to make the mixture distinctly alkaline. Prepare a series of test-tubes for digestion experiments as follows:

A. Place a small quantity of fibrin in a test-tube, adding the alkaline diluted glycerin extract of pancreas until the tube is half full.

B. To another tube containing fibrin add the glycerin extract diluted with water, alone, and without the addition of the alkali.

C. To another portion of fibrin add glycerin extract of pancreas and dilute with 0.1-per-cent HCl.

D. To another portion of fibrin add some glycerin extract which has been boiled, and dilute with 0.1-per-cent sodium carbonate.

E. To another fibrin portion add 0.1-per-cent sodium carbonate, alone.

Prepare a second series of tubes in the same way, substituting commercial pancreatin for the glycerin extract.

Place all these tubes in a water-bath at a temperature of 38° C. From time to time make an observation of the changes which may be going on in the various tubes. After a half-hour, in which tubes

has solution of the fibrin taken place? At the end of an hour, what is the condition of the fibrin in the various tubes?

In those tubes in which digestion of the fibrin has taken place, what difference can be made out, by direct observation, between the action of pancreatic and gastric juice in their attack upon proteids?

Filter the contents of those tubes which have shown digestive change and test for albumins, proteoses, and peptones.

Place some of the filtrate in a dialyzer, and the following day test the fluid surrounding the dialyzer for proteoses and peptones.

5. Amylolytic Action of Pancreatic Juice.—(*a*) Make up some starch paste. Test it with Fehling's solution to make sure of the absence of a reducing sugar. Mix some of this paste with dilute pancreatic extract of neutral reaction. Label this tube A.

In a second tube of starch paste, B, place pancreas extract of alkaline reaction.

To a third tube of starch paste add pancreas extract made acid with 0.1-per-cent HCl. Label this tube C.

To a fourth portion of starch paste add 0.1-per-cent HCl alone. Label D.

To a fifth portion add strong hydrochloric acid. Label E.

To a sixth portion add 0.1-per-cent sodium-carbonate solution. Label F.

Place all these tubes in the water-bath at 38° C. In ten or fifteen minutes test for reducing sugar in all the tubes, first carefully neutralizing those of an acid reaction. Record results. In which tubes has starch digestion taken place? What is the starch-digesting enzyme of the pancreatic secretion? What is the optimum reaction of the digesting medium?

(*b*) Mix some starch paste with pancreatic extract which has been previously boiled. Place in the warm water-bath and note results. What is the effect of high temperature on the enzyme? Is this effect common to all enzymes?

6. Lipolytic Action of Pancreatic Juice.—Mix a small quantity of fresh butter in a test-tube with glycerin extract of pancreas

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and 0.1-per-cent sodium carbonate. Place the mixture in the warm water-bath. What change occurs in the butter? After a time can you detect the odor of butyric acid? If so, what is its significance?

7. Action of Bile.—You will be supplied with ox-bile. What is its reaction?

(*a*) Test for bile pigments as follows (Gmelin's reaction): To a little bile on white porcelain add a few drops of fuming yellow nitric acid. Note the changes in color from green to blue, yellow, and brown-yellow. The test may also be done by placing a drop of bile on white filter paper and bringing a drop of the acid in contact with it. Color rings will be formed at the junction of the bile and the acid.

(*b*) *Pettenkofer's Test for Bile Acids.*—Mix some ox-bile in a test-tube with a small amount of strong sulphuric acid. Test the temperature of the mixture with a thermometer, adding the acid slowly. The temperature should not be higher than 70° C. or lower than 50° C. Now add a 10-per-cent solution of cane sugar, slowly, drop by drop, stirring with a glass rod. A red coloration indicates the presence of bile acids. This reaction is masked by using an excess of sugar or too high a temperature, since the sugar is decomposed and colors the mixture a dark brown.

(*c*) Mix some fresh butter in a test-tube with a few cubic centimetres of ox-bile. Mix a portion of butter in another tube with bile and pancreatic extract. Place in the warm water-bath and note results.

8. Absorption of Fat.—Starve a cat for twenty-four hours and kill. Open the abdomen and note the condition of the mesenteric lymphatics, the lacteals. Open a loop of small intestine. Scrape off some of the mucous membrane. Tease some of the scrapings, on a slide, with glycerin, after previous immersion in osmic acid ½ per cent for twenty-four hours. Remove the excess of glycerin with filter paper, and, covering the preparation with a cover slip, examine under the microscope. If fat-droplets are present in the epithelium, they will be stained black or dark brown by the osmic acid. Note results.

Feed another cat, after a starvation period of twelve hours, with fatty meat or milk and cream. Kill in ten hours after the meal. Prepare the intestinal mucous membrane, as before, with osmic acid. Also note the appearance of the lacteals. Can you demonstrate the presence of fat in the intestinal epithelium?

VII. Mechanism of Pancreatic Secretion. (Demonstration.)

1. Narcotize a medium-sized dog with morphine sulphate and anæsthetize with ether. Expose and introduce a cannula into the trachea. Expose, isolate, and secure both vagus nerves with untied ligatures. Connect the tracheal cannula with the artificial respiratory apparatus and anæsthetic flask. Through a median abdominal incision expose the duodenum and head of the pancreas. Slit the duodenum longitudinally for an inch or more in the neighborhood of the head of the pancreas. Place a cannula in the larger pancreatic duct through its duodenal opening, on a level with the lower border of the pancreas. Connect this with a long glass tube, bent so as to pass over the edge of the abdominal wound. Fill this tube with physiological salt solution.

To the rubber membrane of a transmitting tambour cement a light aluminum shovel-shaped lever. Arrange the transmitting tambour in connection with a recording and a slow drum. Bring the end of the pancreas-cannula tube over the shovel lever of the tambour. Each drop from the end of the cannula will then depress the lever of the transmitting tambour, and this will be transmitted to the recording tambour and a record will be written on the paper of the revolving drum.

During the continuation of the experiment the exposed abdominal viscera should be protected by cotton pads soaked in warm 0.8-per-cent NaCl solution.

After completion of the operative procedures, note the rate of flow of pancreatic juice for a period of ten or fifteen minutes. Now inject into the duodenum or jejunum 30 c.c. of a 0.4-per-cent HCl

solution. Note the rate of pancreatic secretion for another period of fifteen minutes.

Allow ten minutes' rest. Then divide both vagus nerves and stimulate with a weak tetanizing current. Note any change in the rate of pancreatic flow.

Repeat the injection of the acid. Is the same effect produced that occurred after the first injection?

2. Action of Secretin (Bayliss and Starling).—That the chief stimulus to the elimination of pancreatic secretion is a chemical one was demonstrated by Bayliss and Starling. They have found that there is a substance present, in the mucous membrane of the upper part of the small intestine chiefly, which they have termed prosecretin. This, in the presence of hydrochloric acid, or other acids to a less extent, is changed to another substance, secretin, which is absorbed, passes to the pancreas, and there acts as a stimulus to the elimination of pancreatic juice.

An active secretin extract is prepared as follows: Cut out a dog's duodenum and jejunum. Slit open the bowel. Wash thoroughly with water. Scrape off the mucous membrane. Grind this with sand and a little 0.4-per-cent HCl in a mortar. Add three times its volume of 0.4-per-cent HCl and allow to stand for fifteen to twenty minutes. Bring to a boil in a porcelain capsule, and while boiling neutralize and render slightly alkaline with strong NaOH. Acidify slightly with acetic acid, strain through muslin, and filter.

Isolate and introduce a cannula into the central end of the femoral vein of the dog of experiment 1. While a record of flow of the pancreatic juice is being taken, introduce into the vein 5 c.c. of the secretin extract prepared as described above. Note the result on the rate of elimination of the pancreatic secretion.

Repeat the injection several times, allowing a period of rest between injections.

CHAPTER VII.

INTERNAL SECRETIONS.

I. Liver, Glycogen.

1. Select a well-nourished rabbit. Kill quickly by a sudden blow upon the back of the neck in the region of the medulla. As speedily as possible, open the abdomen and remove a portion of the liver. Cut this into small pieces. Place some of these pieces in boiling water; and place one piece immediately on the holder of a freezing microtome and freeze the piece.

(*a*) Make a number of sections with the freezing microtome, and as soon as cut place in Lugol's solution containing iodine and potassium iodide. Allow the sections to remain in the staining solution for two or three minutes. Wash in water to remove excess of the iodine solution. Mount in glycerin and examine under the microscope.

The glycogen present in the liver cells will be stained a mahogany red by the iodine. Compare this reaction with that given by dextrin with iodine.

(*b*) Allow some of the rabbit's liver to remain in the incubator at body temperature for an hour or longer. Cut frozen sections of a piece of this and treat with iodine as before. Is there any glycogen reaction?

(*c*) Grind some of the incubated liver with sand in a mortar. Add two or three volumes of water. Allow to stand for several minutes. Strain through muslin and filter through paper, and test for reducing sugar with Fehling's solution.

(*d*) To prepare glycogen, take the pieces of liver which have been immersed in the boiling water, grind them in a mortar with

fine sand, return to the capsule, and boil again for a short time to make certain that the diastatic power of the liver has been destroyed. Add ten volumes of water slightly acidulated with acetic acid. Strain through muslin. To remove the proteids, concentrate the liquid to a third its volume and add alternate drops of HCl and potassium mercuric iodid until precipitation ceases. Filter off a little of the liquid and test the filtrate for proteids. If there are none present, strain all of the liquid through muslin and filter through paper. To the filtrate add two volumes of alcohol, stirring well. Allow the precipitate, glycogen, to settle, decant off the supernatant liquid, filter the residue, and wash with dilute alcohol. Transfer the residue to a beaker, cover with absolute alcohol, and set aside for an hour. Remove the alcohol and dry the residue between folds of filter paper.

To some of the glycogen thus prepared add 25 c.c. of water and warm gently. A solution is formed. Compare the appearance of this solution with that of soluble starch.

Test a little of the glycogen solution with iodine and KI. Note the color reaction. Does it disappear upon heating? If so, does it reappear upon cooling?

Test some of the solution of glycogen with Fehling's reagent. Is there any reduction of the copper salt brought about?

To a little of the glycogen solution add some saliva. After a few minutes test with Fehling's reagent for reducing sugar.

II. Pancreatic Diabetes.

Effect of Removal of the Pancreas on Carbohydrate Metabolism.— Collect the urine of a medium-sized dog and test for reducing sugar with Fehling's solution. In all probability none will be found. Starve the dog for twelve hours. Inject subcutaneously 0.12 gram of morphine sulphate. Place dog, back down, on the operating-board and continue the anæsthesia with ether. Prepare sterile medium and heavy silk suture material. Sterilize a number of absorbent cotton pads in physiological salt solution. Sterilize instruments by steam or by boiling.

Clip and shave the hair over the midabdominal region and wash the skin with corrosive sublimate, 1 to 1000, followed by alcohol. Clean the hands by thorough scrubbing and immersion in the corrosive-sublimate solution.

Open the abdomen by an incision in the median line. Locate the head of the pancreas at the duodenum and the tail at the spleen. Carefully separate the pancreas from the omentum and mesentery and from the duodenum, tying the pancreatic ducts and all arteries and veins with double ligatures and cutting between. Having controlled hemorrhage in this way, remove the pancreas *in toto*.

Bring the peritoneum and abdominal muscles together with one row of interrupted sutures and close the skin wound with a second row of sutures. Dry the wound with alcohol and ether and paint with collodion.

After the animal has recovered from the anæsthetic and the immediate shock of the operation, collect the urine every six hours and make quantitative estimations of the sugar by Fehling's method. Also, carefully note the condition of the dog, as to loss of weight, temperature, appetite, weakness, convulsions, etc. The diet may be the same as that given previous to the operation. Make an estimate of the carbohydrates, proteids, and fat of the diet.

If the dog survives more than forty-eight hours after the operation, the diet may be changed to pure proteid and a known amount of raw pancreas given with the food.

Immediately after the death of the animal, the liver should be removed and tested for glycogen as described for experiment I.

III. Thyroid.

1. Ablation of the Thyroid in Dogs.—This is best done in two operations. At the first operation one lobe of the thyroid is removed, and at the second the other lobe.

Select a young dog. Record weight. Place under morphine narcosis. Shave and scrub skin of the neck and wash with a bichloride solution, 1 to 1000. The operation should, of course, be

done with strict asepsis. The instruments should be boiled, ligatures sterilized, and hands cleansed and soaked in the bichloride solution.

Expose the trachea through a median cervical incision, carrying the incision as far as the thyroid cartilage. Pull aside and separate, by blunt dissection, the longitudinal neck muscles from the thyroid lobe of one side. This is seen as an oval, reddish mass. With blunt hooks and scalpel handle separate it from its attachments. Tie all blood-vessels with two ligatures and cut between. The thyroid branch of the carotid artery should not be tied too near its origin, since there is danger of the ligature slipping later, and secondary hemorrhage. If an isthmus is present connecting the two lateral lobes, this also should be removed.

The wound is now cleansed with sterile 0.8-per-cent NaCl solution, dried, and closed by two rows of interrupted silk sutures, one to draw the muscles together over the trachea, the other to approximate the skin. Cover the wound with a thin layer of sterile absorbent cotton and paint with collodion.

Keep the animal under careful observation for ten days or two weeks, recording the weight daily. At the end of this time, repeat the operative procedure and remove the remaining thyroid lobe. Keep under careful observation, recording the weight daily, taking the temperature per rectum, and counting the red and white corpuscles of the blood. Note all symptoms and keep a careful record until death occurs. If the animal does not die or show the characteristic symptoms of thyroid removal, all the thyroid has not been removed or there are accessory thyroids present. This may be determined at autopsy later.

At autopsy the condition of all the organs should be determined and the field of the operation examined to see if the thyroid removal was complete and if accessory bodies are present. These are sometimes found in the neck region near the thyroid lobes proper, or in the mediastinum. If found, they should be hardened, embedded, sections cut, stained, and examined for thyroid structure

2. Thyroid Feeding after Thyroid Removal.—Remove the thyroid of another dog in the same way as in experiment 1, and at about the same time, so that the symptoms of the two animals may be compared. The second dog should be fed, after the complete removal of the gland, with fresh sheep's thyroids, or thyroid extracts may be mixed with the food.

What are the symptoms of thyroid removal in the dog? How do they compare with those stated to occur in similar conditions in man? How do they compare with the symptoms of thyroid disease in man? Has thyroid feeding any effect in alleviating the symptoms following thyroid removal in the dog?

If the dog fed with thyroids survives, it should be killed later and careful search made for accessory bodies.

IV. Suprarenal Glands.

1. Ablation of the Suprarenal in the Rabbit.—*Demonstration.*—Select a large, well-nourished rabbit. The Belgian hare serves well for the purpose. Starve for twenty-four hours. The operation is done in two stages, one suprarenal being removed at the first operation, and the other two weeks later. The left suprarenal is removed most readily by the abdominal route. The right suprarenal is reached best by the dorsal route without entering the peritoneal cavity.

Inject under the skin 9 cgm. of morphine sulphate. Strap the rabbit, back down, upon the rabbit-board. Clip and shave the hair from the midabdominal region. Wash with bichlorid. Sterilize instruments by boiling. Sterilize absorbent cotton pads in 0.8-per-cent NaCl solution.

Beginning just below the xyphoid appendix of the sternum, make an incision in the midabdominal line, through the skin, fascia, and peritoneum. The length of the incision should be about three inches. Cover the intestines and hold them back with absorbent cotton pads wrung out in hot salt solution. Let an assistant hold back the edges of the abdominal wound with retractors. Locate the left kidney. Follow the renal vein to its junction

with the inferior vena cava. A little above the angle formed by these two veins and closely hugging the inferior vena cava, the left suprarenal capsule will be seen.

This is a yellowish-white oval body, lying behind the peritoneum. Its blood supply is variable. There are generally one large artery and vein entering the hilum of the gland, and these must be tied before the gland is excised.

With small blunt hooks and a blunt-pointed seeker tear through the overlying peritoneum. Separate the gland from its surroundings slowly and carefully, being especially careful to avoid injury to the inferior vena cava.

Remove all cotton pads from the abdominal cavity. Bring the muscles and peritoneum together with one row of silk sutures, and the skin with another row of sutures. Cover the wound with some antiseptic powder and apply a gauze bandage. Place the rabbit in its cage and observe it carefully twice a day for the first two days and then make daily observations. At the end of two weeks the remaining gland may be removed. In the mean time make an extract of the gland as described below.

2. Weigh the suprarenal which you have removed. Grind it in a mortar with fine clean sand and a little 0.7-per-cent NaCl solution. Add of the salt solution enough to equal ten times the weight of the gland. Transfer to a tightly stoppered bottle and place in the incubator at 30° C. for fifty-six hours. Strain through muslin and filter through paper. One part of this extract will be equivalent to $\frac{1}{10}$ of the fresh gland.

3. (*a*) Prepare another rabbit for a blood-pressure experiment. Isolate both vagi and secure them with untied ligatures. Prepare one jugular vein for injection. Take a normal blood-pressure tracing. While the tracing is being recorded, inject into the jugular enough of the suprarenal extract to be equivalent to $\frac{1}{10}$ of the fresh gland. Note the effect on the heart-beat and on blood pressure. What is the duration of the effect of the injection?

(*b*) Repeat the injection, and immediately after cut both vagus

nerves. Compare the effect of the second injection upon heart-beat and blood pressure with the result of the first injection, when the vagus nerves were intact.

(*c*) Repeat, using in place of the extract of the rabbit's suprarenal, 1 c.c. of a 1 to 10,000 solution of adrenalin chlorid.

The effect of the suprarenal extract upon skeletal muscle has already been shown under Muscle-nerve Physiology.

4. Remove the second suprarenal from rabbit 1 as follows: Place the animal under morphine and ether. Tie, belly down, upon the operating-board, placing a pad under the abdomen for the purpose of bringing the viscera into the field of the operation. Shave and cleanse the skin of the right flank. Beginning at the lower border of the ribs, make a longitudinal incision through the skin and fascia of the flank about three inches long and about two inches from the spinous processes of the vertebræ. Cut through the lumbar aponeurosis of the abdominal muscles, and separate these from the heavy spinous muscles. Locate the kidney. Let an assistant, standing on the right side of the operating-table, hold the kidney down and to the right with one finger, and with two fingers of the other hand pull the lateral margin of the wound up and out. The operator should stand to the left of the subject. Follow up the renal vein as before, until the right suprarenal body is seen. Separate this from its attachments in the same way as was done in removing the left suprarenal. Remove the gland entire, or, if this cannot be done with safety, remove as much as possible and crush the remainder with forceps. Sew up the wound with two rows of sutures and return the rabbit to its cage. Since death frequently occurs within the first twelve hours following complete removal of the suprarenals, the operation is preferably done in the morning, so that the animal may be under observation all day. If the operation has been successfully performed with the minimum of shock and hemorrhage, the animal should regain consciousness. Make careful note of all symptoms from the time of the operation until death occurs.

At the autopsy examine all the organs and make careful search for accessory suprarenals and for any stump remaining after removal of the glands.

Compare the symptomatology of the rabbit minus suprarenals with the symptomatology of Addison's disease in man.

CHAPTER VIII.

RESPIRATION.

I. Respiratory Movements in Man.

1. Direct Observation.—Let one of the students of a laboratory group, preferably one of slender build, strip to the waist. Let him sit straight on a stool with the arms and hands symmetrically placed at the sides, and both sides of the chest evenly illuminated.

(*a*) Observe first whether or no the two sides of the chest are of equal size and the respiratory movements equally prominent on the two sides. Note the increase in the lateral and antero-posterior diameters of the thorax in inspiration. Note the movements of the abdominal wall in inspiration and in expiration. Note the changes in the intercostal spaces during the respiratory movements.

What muscles seem to be involved in quiet inspiration? in quiet expiration? In which part of the thorax is the lateral diameter enlarged most, during quiet inspiration? What are the movements of the ribs during expiration and inspiration? Demonstrate these movements on the skeleton thorax.

Remembering the attachments of the external and internal intercostal muscles, demonstrate their action on the skeleton thorax by the use of heavy elastic bands attached to the ribs in the same way as the intercostals are attached.

(*b*) Now let the subject of the observation make a forced inspiration, followed by a forced expiration. Compare this with the movements of quiet respiration. What additional muscles are involved in inspiration? in expiration?

(*c*) By means of some form of pneumograph or stethograph record the respiratory movements. This may be conveniently

done by strapping to the chest a rubber bulb connected with a recording tambour whose lever is allowed to write on a medium fast drum. A time tracing in seconds should also be taken.

First record the respiratory movements while the subject is in the recumbent position. Take the pulse rate at the same time. Allow the subject to sit, and record the respiratory movements again. Repeat the record with the subject standing. Compare the rate and character of breathing, together with the pulse rate, in the three postures.

Compare the inspiratory phase with the expiratory phase. What is the ratio between the two? Under what pathological conditions may this ratio be disturbed and in what way? What is the relation between rate of heart-beat and respiratory rate? Under what pathological conditions is this ratio disturbed?

(*d*) Let the subject take some form of exercise for a few minutes, such as running up a flight of stairs. Record respiratory movements again. Compare with other tracings. Observe rate of heart-beat.

(*e*) While a tracing of the respiratory movements is being taken let the subject take several swallows of water in rapid succession. While the swallowing is going on, what is the effect on the respiratory movements? To what is this effect due?

2. Respiratory Sounds. Auscultation.—In order to distinguish abnormal respiratory sounds in pathologic pulmonary conditions it is necessary to be familiar with the normal. There are normal individual variations which must also be taken into account. It is therefore well to examine a number of normal chests.

(*a*) *Vesicular Breathing.*—With a stethoscope, first listen, during quiet respiration, at about the fifth or sixth right intercostal space. A sound more distinct and of longer duration on inspiration will be heard. The character and quality of this sound is hard to describe. It is best compared, perhaps, to the sound made when the leaves of a tree are stirred by a light breeze.

What is the ratio of inspiratory sound to expiratory sound? Compare this with the ratio of inspiration to expiration.

(*b*) *Bronchial Breathing.*—With a stethoscope listen to the breathing over the trachea and over the second costal cartilage on the right side. The latter position is at the bifurcation of the right bronchus. The sounds heard here are due to the passage of air through a tube and in part over the mouth of a tube. This is a fair example of normal bronchial breathing. Compare with vesicular breathing. Should bronchial breathing be heard where you listened for vesicular breathing? What change in the lung might give rise to bronchial breathing?

(*c*) Listen over the right lung during forced respiration. How do the respiratory sounds differ from those of quiet respiration?

(*d*) Now go over the chest systematically, first one side and then the other, comparing the two. Start at the supraclavicular space of one side, then listen in the same region on the other side, and so on over all of the anterior, lateral, and posterior aspects of the thorax. Note any differences in the sounds in the different regions of the chest either in intensity, or duration, or character. Explain.

3. Palpation. Vocal Fremitus.—Place the same hand successively over the various regions of the chest both in front and behind, alternating sides, so as to compare one region with its fellow of the opposite side. While the hand is applied to the chest wall let the subject say "twenty-one" or some other number. The vibrations of the chest wall during phonation are distinctly felt by the hand. There are pathologic variations of this, either in an accentuation of the fremitus on one side as compared with the other, or in a diminution of the fremitus.

Listen again over the various regions of the chest while the subject counts "one, two, three." The sound will be transmitted to the ear, but more as a murmur, and not as distinctly enunciated syllables. In certain pathologic conditions the spoken or whispered word is very distinctly heard, as if it were spoken directly into the end of the stethoscope. What physical changes in the lung might cause this condition?

4. Percussion.—Laying the middle finger of the left hand between the ribs, in the intercostal spaces, and using the bent mid-

dle finger of the right hand as a hammer, percuss the entire chest wall. Explain the variations in the percussion note over various regions. Map out the heart and the liver.

5. Chest Measurements.—(*a*) Let a student strip to the waist. Measure the chest circumference at the axillæ at the end of quiet inspiration and at the end of quiet expiration. Repeat this measurement at the level of the end of the sternum. Repeat both measurements, in forced inspiration and expiration. Record results.

(*b*) In the same individual measure the antero-posterior thoracic diameters at the junction of the first and second pieces of the sternum and at the end of the sternum, during inspiration and expiration, both quiet and forced. Measure the changes in the lateral diameters in the same way. A pair of long, graduated calipers serves for the purpose of making the measurements. Record results.

For purposes of comparison, measure the length of the trunk from the "vertebra prominens" to the level of the chair upon which the subject is sitting. Repeat these measurements on other students and keep a record for each individual.

6. Respiratory Capacity.—This is usually determined by some form of water spirometer. A long, narrow cylinder, graduated in cubic centimetres, is filled with water and inverted over water in another cylinder. An air tube passes through the second cylinder to the top of the first. The first cylinder is counterpoised by weights and pulleys, so that when air is forced into it the water is displaced, the cylinder rises, and the amount of water displaced by air can be read off on the attached scale.

(*a*) *Calibration of Spirometer.*—The air cylinder of the spirometer should be calibrated before using. This may conveniently be done in the following way: Fill the cylinder with air; note the position of the pointer on the scale; place the outlet air tube under a graduated 1000 c.c. cylinder, filled with water and inverted over water, in a large pan or tub; slowly depress the spirometer cylinder until the pointer has passed five or ten spaces of the scale, and read off the amount of water displaced from the graduated cylin-

der in the tub; record and repeat the operation until all the spirometer scale is estimated in terms of cubic centimetres of the testing graduated cylinder.

(*b*) *Tidal Air.*—With the pointer of the spirometer at the zero mark of the scale, expire into the spirometer cylinder after an ordinary inspiration. The amount of expired air recorded is an approximate indication of the tidal air, *i.e.*, the amount that passes in and out of the lungs during quiet respiration.

(*c*) *Supplemental Air.*—Repeat the spirometer record, taking a normal quiet inspiration, and then forcing as much air out of the lungs as possible. Read the record on the spirometer scale. Subtract the reading of (*b*) from the latter reading. The difference is the so-called supplemental or reserve air. This is the amount of air which remains in the lung after a quiet expiration and which may be expelled by a forced expiration. After this is expelled, air still remains in the lung which cannot be forced out. This is the *residual air*. The air which can be inspired in addition to the ordinary inspiration is known as the *complemental air*.

(*d*) *Vital Capacity.*—Take the deepest possible inspiration, and empty the lungs as completely as possible into the spirometer cylinder by a forced expiration. The record obtained indicates the full pulmonary capacity minus the residual air, and is equal to the sum of the tidal air, complemental air, and supplemental air. This is known as the *vital capacity*.

Record the vital capacity of each member of your group, and compare with the various chest measurements already taken.

7. Cardio-pneumatic Movements.—The changes in volume of the heart during systole and diastole cause corresponding changes in the capacity of the thorax and consequently of the lungs. The inspiratory and expiratory movements caused by the heart-beat may be demonstrated in the following manner: Bend one end of a medium large piece of glass tubing into the form of a U. Fill the bend of the U with a little water colored with eosin. Place the end of the horizontal limb of this tube in one nostril. Close the other nostril with the finger and keep the mouth closed. Hold the

breath. The fluid in the U will move synchronously with the heart-beat. At each systole there will be an inspiratory movement, and at each diastole an expiratory movement.

II. Pulmonary Pressure.

Place a rabbit under morphine narcosis. Anæsthetize lightly with ether. Place, back down, on rabbit-board. Expose the trachea through a median cervical incision. Introduce a tracheal cannula. Connect this, through a T-tube, with the proximal end of a mercury manometer. Place a clip on the rubber tubing, leading from the T to the manometer, so that it can be either opened or closed. At the beginning of an inspiration, open the manometer clip and close the air-inlet limb of the T. At the height of inspiration, close the clip on the manometer tube and open the air-inlet tube. The inspiratory negative pressure may then be read off from the manometer scale. A tracing of the respiratory movements may be obtained in this way by partially occluding the air-inlet tube. Close the air-inlet tube during several respiratory cycles. Note the positive pressure of the expiratory phase and the negative pressure of the inspiratory phase. After a few respiratory efforts the animal will begin to struggle because of asphyxia. When this occurs, note the change in the character of the respirations and the great difference between inspiratory and expiratory pressure. In quiet respiration, is the positive pressure in expiration much above the atmospheric pressure?

III. Intrathoracic Pressure.

Using the same rabbit as in the previous experiment, connect the proximal limb of a manometer, through a piece of pressure tubing, with a small glass tube. Make a small incision through the skin over the fourth intercostal space on the right side. Make a very small nick through the intercostal muscles and force the glass tube through this opening. The tube should fit so tightly that there will be no leakage of air around it. Note the movements of the mercury in the manometer with inspiration and expiration. Com-

pare them with the changes of intrapulmonary pressure. In quiet breathing, what is the constant condition of pressure in the thorax? How does this pressure change with inspiration and expiration? Explain.

Now close the tracheal cannula and induce asphyxia. Note the variations in intrathoracic pressure during the violent respiratory efforts which occur in this condition.

For the influence of the respiratory movements on the blood pressure and heart-beat, refer back to your blood-pressure tracings, and study again the respiratory waves of the tracings. When is the intrathoracic pressure lowest? when highest? What effect would changes of pressure within the thorax have upon the pressure of blood in the large arteries and veins and in the heart itself during diastole?

IV. The Vagus Nerve in Respiration.

The same rabbit that was used in the previous experiments may be used for this. Continue the dissection of the neck region very carefully, isolating both vagus nerves, and both superior laryn-

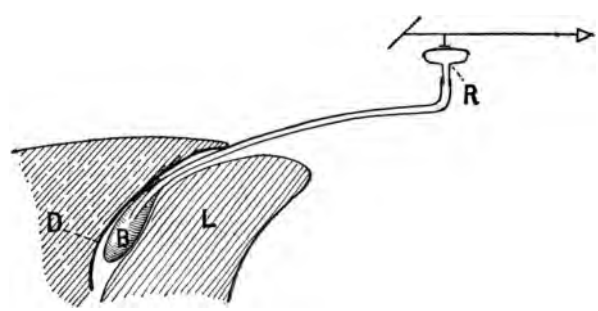

FIG. 37.—To Record Movements of Diaphragm. *R*, Recording tambour; *B*, rubber bulb, connected with recording tambour; *D*, diaphragm; *L*, liver.

geals. Make an incision through the abdominal wall below the xyphoid appendix of the sternum, just large enough for the introduction of a catheter over the end of which a collapsed rubber balloon is tied. Pass this up between the diaphragm and the liver, on

the right side. Connect the catheter with the recording tambour (see Fig. 37), and the movements of the diaphragm will be recorded upon a drum revolving at medium speed.

(*a*) First take a tracing of the normal movements of the diaphragm. Take a time tracing in conjunction with the respiratory tracing. Note the rate of the respirations in the rabbit. These may be influenced by the morphine which has been previously given the rabbit.

(*b*) While the tracing is being taken, tickle the rabbit's nose with a feather. Is there any effect on the respiratory movements? What nerve has been stimulated?

Note the movements of the rabbit's nostrils with inspiration and expiration. These movements continue, even though breathing is no longer taking place through the nose, but through the tracheal cannula. Other associated movements you have already noted, *e.g.*, the opening and closing of the glottis.

(*c*) Pinch the skin or pour a little ether on the shaved abdominal surface. What effect has cutaneous stimulation on respiratory movements?

(*d*) Now tie one vagus with two ligatures and cut between. Mark on the tracing the time at which the vagus was tied and cut. Note any change in the respiratory movements occurring at the time of tying and cutting the nerve or following this operation. If the respiratory rhythm has been disturbed, does it return to normal, after a time?

(*e*) Allow five or ten minutes to elapse and then stimulate the central end of the cut vagus with a weak tetanizing current. What is the effect on the respiratory movements?

(*f*) Stimulate the central end of the cut vagus with a medium strong tetanizing current. Compare this effect with that obtained with the weak stimulation.

(*g*) Apply to the central end of the cut nerve a few crystals of NaCl. Note the change in the respiratory movements. After the effect of this stimulus is sufficiently evident, cut off the small piece of nerve to which the salt has been applied.

(*h*) Stimulate one superior laryngeal nerve with a weak tetanizing current. Note the effect on the respiratory movements. Stimulate with a stronger current and note the effect.

(*i*) Now tie and cut the other vagus nerve. Both vagi are now severed. What is the effect on the rhythm, rate, and depth of the respiratory movements?

(*j*) Stimulate both central ends of the cut nerves with a weak tetanizing current. Note the effect on the rate, rhythm, and character of the respirations. Stimulate with a stronger current. Result?

(*k*) Stimulate both vagi with a weak tetanizing current, and, when the effect of such stimulation begins to show, stimulate both superior laryngeal nerves with a medium strong current. Result?

In all of the above experiments, marks should be made upon the tracings to indicate the operative procedures and their time relation to the tracing; the nerves stimulated and the strength and nature of the stimulus employed.

(*l*) Stimulate the peripheral ends of the divided vagus nerves. Is there any effect on the respiratory movements?

From the above experiments what conclusions can you draw concerning the function of the vagus nerve in relation to respiration? Is the vagus chiefly an afferent or an efferent nerve in relation to the lungs?

The vagus has now been studied, in part, in connection with the circulation, digestion, and respiration. Compare its functions in relation to the three systems. It contains both afferent and efferent fibres for circulation, digestive tract, and respiratory tract. Summarize your knowledge on the subject, secured in part through your own experiments in the laboratory.

V. Innervation of the Diaphragm.

1. Still using the same rabbit that was used in the previous experiments, expose the phrenic nerve of the right side. This may be done in the following manner: Enlarge the cervical incision to

the upper end of the sternum. Pull the sterno-mastoid and other longitudinal neck muscles toward the median line. Pull the skin and other muscles to one side and expose the cervical spinal nerves and the beginning of the brachial plexus. The carotid artery, vagus nerve, and jugular vein should also be pulled toward the median line. Determine the position of the fourth, fifth, sixth, and seventh cervical nerves. Arising by filaments from the posterior divisions of these nerves, a fine nerve fibre will be seen running over the heavy spinous muscles, parallel with the spinal column and disappearing under the clavicle. To make sure that you have found the phrenic nerve, place fine platinum electrodes under this nerve filament and stimulate with medium strong single-induction shocks. The diaphragm recorder which records the movements of the right side of the diaphragm will move with each stimulus.

2. Pass a thread around the upper origin of the nerve. Tie the nerve and sever all connection with the cervical nerves. Note the diminution or complete loss of recorded movement of the diaphragm. The right side of the diaphragm is paralyzed and moves only as it is pulled upon by the side which is still active. Compare the thoracic breathing after the section of one phrenic with the thoracic breathing before the section of the nerve.

Do the two sides of the chest move equally, or is there a difference between the right and the left side?

3. Stimulate the nerve with single shocks from an inductorium, and take a tracing on the drum.

4. Enlarge the abdominal opening and pull down the abdominal viscera so that the movements of the diaphragm may be observed directly. Note the movement of the left side, whose nerve supply is still intact. Note the lack of motion on the right side and the position of this enervated side of the diaphragm during inspiration and expiration.

5. Expose and cut the left phrenic nerve. Both sides of the diaphragm are now paralzyed. Note the change in the respiratory movements. If the rabbit is young there will be great diffi-

culty in breathing, amounting to distinct dyspnœa. Note the increase in thoracic breathing.

Explain the difficulty in breathing following the paralysis of the diaphragm. Is it due simply to the loss of the active movements of the diaphragm in enlarging the vertical diameter of the thorax?

6. Place both phrenic nerves on electrodes from the inductorium. Stimulate with a medium strong tetanizing current, interrupted about thirty times per minute. Is the dyspnœa relieved? Does the rabbit cease attempts at thoracic breathing?

7. Close the trachea by means of an artery clamp and kill the rabbit by asphyxiation. Note all phenomena connected with death from this cause. After breathing has ceased, open the thorax and observe the heart. Is this still beating? Note the color of the blood. Note difference between the two sides of the heart. Excise the heart. Does the heart-beat recover for a short time after excision?

VI. Effect of Blood Temperature on Respiration.

1. Heat.—Narcotize a rabbit. Place, back down, on the rabbit-board. Through a median cervical incision expose both carotid arteries and isolate the vagus nerves. Arrange the apparatus for recording the movements of the diaphragm. Isolate as much of each carotid as possible. Separate the artery from the nerves running with it, by several layers of paper. Tie each carotid, gently, to a small tubing running parallel to the artery. One end of this tubing is connected to a rubber outlet tube. The other end is connected to a rubber inlet tube. The inlet tube leads from a bottle filled with water kept at a temperature of 40 C., by immersion in a water-bath. This bottle is elevated sufficiently to give a constant flow of warm water through the tubes in contact with the arteries. The blood passing through the carotids is therefore warmed two or three degrees above the normal.

Set up the arrangement as above described. First take a normal respiratory tracing. Then, while a tracing is being taken, let the warm water run through the tubing and note the effect upon res-

piration. Change in the pulse rate may also be observed by watching the pulsation of the carotid arteries or by feeling the cardiac impulse on the chest wall. How are the respirations affected when the blood going to the brain is warmed above the normal? How is the heart-beat affected? What is the relation between heart-beat, respiratory rate, and rise of temperature?

2. Cold.—Disconnect the warm-water bottle from the tubes in contact with the carotids, and substitute a receptacle containing ice water. First, let the respiratory rate return to normal. Then while a tracing is being taken, allow the cold water to flow through the tubing in contact with the arteries. Note the effect upon the respiration and the heart-beat. Compare the tracing obtained through the cold application with that obtained when heat was applied.

What is the effect of warmed blood upon the respiratory centre? of cold blood upon the respiratory centre?

VII. Effect of Anæmia upon the Respiratory Centre.

1. Using the same animal as in the previous experiments, clamp both carotids with artery clips. Is there any change in respiration following the occlusion of both carotids? If so, does this changed respiration continue or does it soon return to the normal? Explain. Does occlusion of the carotids materially or permanently diminish the blood supply to the brain? What is the collateral circulation?

What would be the only way completely to cut off the blood supply to the brain?

2. Tie both carotids as high up as practicable. Introduce a glass cannula into each artery. Take a normal respiratory tracing. While this is being taken, open the clips on both arteries and allow the animal to bleed to death. Collect the blood in a graduated cylinder and record the phenomena observed after the loss of each additional 10 c.c. of blood. How much blood is lost before the respirations are affected? In what way are the respirations affected as hemorrhage continues?

Note also every few seconds the condition of the various reflexes. Among these try the reaction of the pupil to light, the conjunctival reflex, the reflex upon tickling the nares, and cutaneous reflexes. Note the time at which each one of these reflexes disappears. Note time when struggling begins. How do these respiratory phenomena differ from, and resemble, those of asphyxia?

VIII. Respiratory Centre.

Narcotize a rabbit lightly with morphine. Anæsthetize with ether. Expose the trachea and both carotid arteries through a median cervical incision. Introduce a cannula into the trachea and tie both carotids.

Change the position of the animal so that it lies on the rabbit-board belly down. Make an incision in the median line through the integument of the skull from the root of the nose to the occiput. Pull the skin flaps to one side, exposing the parietal bones of the skull. Make two trephine openings, one through the parietal bone of each side, enlarging the openings with cutting forceps, until the entire skull cap is removed. Be careful in crossing the median line not to injure the longitudinal venous sinus.

1. Open and lay back the dura on each side, thus exposing both cerebral hemispheres. With a blunt spade or scalpel handle crush both cerebral lobes. Control the hemorrhage by packing with cotton moistened with adrenalin 1 to 10,000, or use the actual cautery.

Observe the respiratory movements before, during, and after this operation. Do respirations continue after the removal of the cerebrum? Is the controlling respiratory centre located, therefore, in the excised portion of brain?

2. Continue the median dorsal incision until all the cervical vertebræ are exposed. Continue the removal of the skull cap until the cerebellar hemispheres are exposed. Remove the cerebellar hemispheres in the same way that the cerebral lobes were removed.

Do the respiratory movements cease in the absence of the cere-

bellum? Is the controlling respiratory centre, therefore, located in the cerebellum?

3. Divide the upper part of the medulla by an incision between the atlas and skull. Do the respiratory movements continue?

4. Divide the cord on a level with the origin of the seventh cervical nerve. In the rabbit the respirations are altered but little, since in this animal breathing is chiefly diaphragmatic. By this section the thoracic muscles are cut off from the respiratory centre, but the innervation of the diaphragm is still intact. Impulses are still carried to the centre from the periphery by the intact vagus nerves. Other afferent impulses are in the main cut off from the centre by its isolation from the brain above and the cord below.

5. Expose and cut both vagus nerves in the neck region. Note the change in the character of the respiratory movements and the disturbance of the respiratory rhythm. Are the respiratory movements which still continue sufficient to sustain the life of the animal?

Allow the first stages of asphyxia to occur. Then revive the animal with artificial respiration continued for a short time.

6. Place the central ends of the divided vagi on electrodes from an inductorium set up for medium strong tetanizing currents. Stimulate the vagi with this current, interrupted about thirty times per minute. Stop the artificial respiration. Is the respiratory rhythm re-established? Compare the results obtained from this experiment with those obtained through section and stimulation of the vagi in former experiments. What conclusions can you draw concerning the location of the respiratory centre and the regulation of its rhythmic activity?

IX. Condition of Lung Following Section of Both Vagi.

Narcotize and anæsthetize a rabbit. Under aseptic precautions expose and cut both vagus nerves in the neck region. Sew up the wound and return the animal to its cage. Make careful observations of the subject until death occurs, which will generally be within forty-eight hours. Determine the cause of death at au-

topsy. Observe particularly the condition of the lungs. Save pieces of the lung tissue for hardening, embedding, and sectioning. Stain sections with hæmatoxylin and eosin and study with the microscope.

What pulmonary condition follows section of the vagus nerves and how is it brought about? Save these sections for comparison with sections of human lung showing areas of lobar and lobular pneumonia.

X. Artificial Respiration.

The student should be familiar with at least one good method of artificial respiration for use in emergencies. One of the best methods for this purpose is the so-called Sylvester's method.

It consists in imitating, so far as possible, the normal respiratory movements. In applying this method the operator should assure himself that the respiratory passages of the subject are free. The subject is placed upon his back, the shoulders being elevated by some support placed beneath them. The head should be on a lower level than the feet.

The operator should stand at the head of the subject and, grasping the wrists, flex the forearm upon the arm and press both arms firmly against the sides of the chest, pressing down and in on the chest at the same time. This motion forces air out of the lungs. When the pressure upon the chest is released, the thorax through its own elasticity rebounds to its original capacity, and air, by this motion alone, is drawn into the lungs. The thoracic diameters are still further increased by the second part of the operation.

This consists of extending the arms and pulling them above the head, giving an extra tug when the position of full extension has been reached. The accessory respiratory muscles, mainly the pectorals, are thus put on the stretch and in their turn pull up and out on the upper part of the thorax.

After this has been accomplished, the first position is again assumed and expiration is brought about. This alternate forced expiration and inspiration are continued at the rate of fifteen to twenty

per minute, until the subject begins to make respiratory efforts of his own or no doubt remains that life is extinct.

XI. Estimation of CO_2 and H_2O Expired in a Given Time.

The estimation of carbon dioxid and water expired by a small animal in a given time may be conveniently done by each group of students. Absorption tubes, made for the purpose, may be

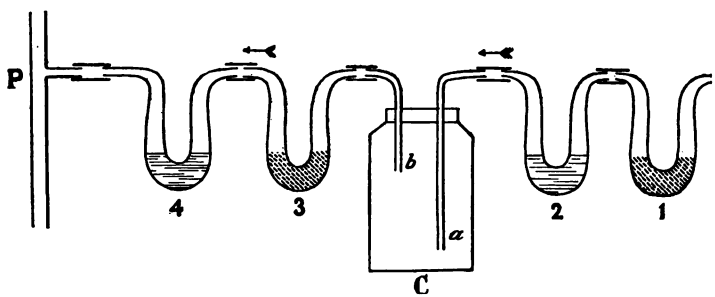

Fig. 38.—Apparatus for Estimating the Water and Carbon Dioxid Eliminated from the Lungs of a Small Animal. Described in text.

bought at a moderate price, as well as the respiratory chamber with air inlet and outlet and tightly fitting stopper. A schematic sketch of the apparatus used is given in Fig. 38.

The chamber *C* is intended for holding some small animal, such as a rat or a mouse. This is tightly stoppered, the stopper being perforated for the passage of two tubes—(*a*) the air-intake tube, which passes nearly to the bottom of the chamber, and (*b*) the air-outlet tube, which begins near the upper part of the chamber. The air-intake tube is connected with two absorption tubes—1, containing pumice stone soaked in sulphuric acid, for absorbing the moisture from the air which passes to the respiratory chamber, and 2, containing soda lime for absorbing the carbon dioxid of the inspired air.

The air outlet tube is also connected with two absorption tubes—3, containing sulphuric acid for absorbing the water of the expired air, and 4, containing a strong solution of sodium hydrate for ab-

sorbing the carbon dioxid of the expired air. This tube also has a bulb containing $CaCl_2$.

Tube 4 is connected with the water pump *P*. Air is drawn through the system of tubes and respiratory chamber in the direction of the arrow.

1. Set up the apparatus as described, and allow the air to be drawn through for fifteen or twenty minutes or until the respiratory chamber has been freed from moisture and carbon dioxid. Now place the mouse in the chamber, stopper quickly, and weigh. Also quickly weigh tubes 3 and 4. Make a record of the weights for use later. Connect up the absorption tubes with the chamber and air pump, and allow a current of air to pass through the system for twenty minutes to one-half hour. Then disconnect and weigh the chamber and absorption tubes again.

The difference between the two weighings of the respiratory chamber indicate the loss, in part, of the animal, in water and CO_2. The difference between the weighings of the absorption tubes indicates their gain in water and CO_2, respectively, and the actual output of the animal in carbon dioxide and water, during the time of the experiment. The difference between the two weighings of the respiratory chamber and the difference between the two weighings of the absorption tubes do not correspond, since what the animal has lost in CO_2 it has partly regained in oxygen.

The gain in oxygen may be roughly determined by subtracting the difference between weighings of chamber *C* from the difference between weighings of tube 4. The ratio between the oxygen absorbed and the carbon dioxid expired is known as the respiratory quotient.

What is the respiratory quotient for the mouse experimented upon?

2. Remove the mouse from the respiratory chamber. Ventilate the chamber again. Prepare and weigh a new set of absorption tubes. Give the mouse some form of exercise, such as moving the treadwheel of a squirrel cage. Place in the chamber and quickly

weigh. Determine the CO_2 elimination for another period equal to the first period in time.

How does the CO_2 elimination of the second period compare with that of the first? What is the difference in the respiratory quotient?

Repeat these experiments, using a cold-blooded animal instead of the mouse. The frog will serve as a type. The mouse is an animal of high respiratory activity. The frog is an animal of low respiratory activity. What is the reason for a higher rate of gas exchange in the mouse than in the frog?

Study the nature of the respiratory movements in the frog. How do they differ in mechanism from those of mammalia? How is respiration carried on in fishes and in gilled amphibia?

What points in common have these three orders of animals as far as respiration is concerned? What points of difference?

A fair idea of the function of the circulation in respiration may be obtained from a study of the circulation through the gill of Necturus.

CHAPTER IX.

EXCRETION.

IT is assumed that the chemical examination of the urine, both for normal and abnormal constituents, has already been done by the student under the direction of the department of chemistry. The chemistry of the urine, therefore, will not be taken up here.

This chapter is limited to an outline of a few experiments dealing with the method of urine secretion and excretion.

1. Movements of the Ureter and Bladder.—Narcotize a rabbit, lightly, with morphine. Anæsthetize with ether, just sufficiently to keep the animal quiet.

Prepare absorbent-cotton pads soaked in hot physiological salt solution for protecting the abdominal viscera after the abdomen has been opened. Open the abdomen in the median line. Continue the incision to the symphysis pubis, so as to expose the bladder.

Note the form of this organ and its relation to the surrounding viscera. If the bladder is full, stimulate it by mechanical irritation or by the application of a tetanizing induced current. Note the character of its contraction. Does it continue to contract and empty itself after the original stimulus has ceased to act?

Collect the urine and save for examination and comparison in color, clearness, specific gravity, reaction, and constituents, with the normal urine of man.

Open the bladder and locate the entrances of the two ureters. Observe these, for a time, for the passage of urine into the bladder.

Trace the left ureter to the kidney. Dissect this out from its bed, so that the kidney, ureter, and bladder are easily observable. Observe the movements of the ureter. What is their nature? How do they compare with the movements of the intestines?

What is the normal direction of the ureter movements? Can the movements be induced in response to a mechanical or electrical stimulus? Are the movements rhythmical or irregular?

2. Urine Flow. Kidney Volume.—Introduce a fine glass cannula into the ureter, near the bladder or through the ureteral opening into the bladder. The speed of urine flow may be recorded by allowing the drops from the end of the cannula to fall upon a lever, made for the purpose and connected with a tambour membrane. This tambour is connected, through rubber tubing, with a second tambour whose lever is arranged to write upon the smoked paper of a slowly revolving drum.

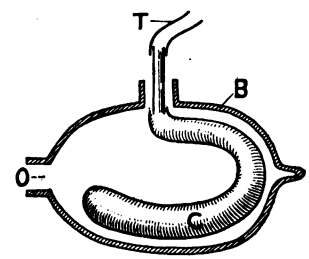

FIG. 39.—Oncometer, Simple Form. *B*, Metal jacket; *O*, opening for kidney vessels; *C*, rubber balloon, inflated with air, partly surrounding the kidney and connected through *T* with a recording tambour.

The changes in volume of the kidney may be determined by means of a plethysmograph arrangement known as an *oncometer*. The oncometer, as generally used, consists of a metal jacket lined with some membrane for enclosing the kidney. There is an opening for the passage in and out of the kidney vessels—artery, vein, and ureter. The space between the membrane and the jacket is filled with oil. This space is connected through tubing with a piston recorder whose lever is arranged to write upon the smoked paper of a revolving drum. In this way a curve of kidney volume is written. A simple form of air oncometer is shown in Fig. 39.

The kidney is partly encased in a rubber balloon inflated with air. The changes in pressure in the balloon are transmitted, through rubber tubing, to a recording tambour or bellows recorder.

The bellows recorder devised by Brodie is far preferable to the tambour as a recorder of volume changes. It can be easily made in the laboratory and consists of two rectangles, hinged with thin

leather, the base being made of vulcanite or wood and perforated for the entrance of the inlet tube. The top consists of a light aluminum frame covered with paper. The sides are made of peritoneal membrane, varnished with a dilute solution of boiled linseed oil to make them airtight.[1]

Place the kidney in the oncometer, cover the exposed abdominal viscera with the warm cotton pads moistened with physiological salt solution, and arrange the recording apparatus for writing on a medium slow drum. Arrange the recorder for urine flow under the kidney-volume recorder. The urine should also be collected for examination, later. Be careful that the ureter, the renal artery, and renal vein are not obstructed by kinks. Keep the ureter from drying by moistening, from time to time, with physiological salt solution.

Observe and record the changes in kidney volume and urine flow for a period of twenty minutes or one-half hour. Is the rate of urine flow constant during this time? Are there any changes in the volume of the kidney? How do urine flow and kidney volume correspond?

3. Blood Pressure and Kidney Volume.—Expose the carotid artery, vagus nerve, depressor nerve, and jugular vein. Introduce cannulæ into the artery and vein. Pass thread loops around the nerves for convenience in handling. Connect the artery with the mercury manometer. Record blood pressure on the same drum used for recording kidney volume and urine flow. Note the correspondence between the changes in blood pressure and changes in kidney volume.

(*a*) Divide one vagus nerve. Stimulate the peripheral end with a tetanizing current sufficiently strong to cause inhibition of the heart-beat. Note the effect upon the volume of the kidney.

(*b*) Allow the blood pressure to recover from the effect of the vagus stimulation. Now stimulate the depressor nerve with a medium strong tetanizing current until a marked depressor effect is obtained. Note the effect on kidney volume and urine flow.

[1] For further details see *Journal of Physiology*, vol. xxvii., p. 473.

(*c*) Allow the blood pressure to return to normal. Allow the animal to inhale a few whiffs of amyl nitrite. Note the effect upon blood pressure, kidney volume, and urine flow.

(*d*) After the blood pressure has again returned to normal, inject into the jugular vein one cubic centimetre of a 1 to 10,000 solution of adrenalin chlorid. What is the effect upon the blood pressure, kidney volume, and urine flow?

(*e*) After the blood pressure has returned to normal, connect the vein cannula with a burette containing warm physiological salt solution. Being careful that there are no air bubbles in the connecting tubes, allow the solution, under low pressure, to run slowly into the vein.

Run in fifty cubic centimetres of the solution. Is there any noticeable rise of blood pressure? Explain. Is there any change in kidney volume or urine flow?

(*f*) Run into the vein fifty cubic centimetres more of the solution. Note any effect upon blood pressure, kidney volume, or urine flow.

(*g*) Repeat the perfusion, using fifteen cubic centimetres of a 1-per-cent urea solution.

4. Intravenous Injection of Dextrose.—Using the same rabbit as in the previous experiment, or a fresh animal if necessary, prepare a 1-per-cent dextrose solution. Warm this to body temperature and slowly inject twenty cubic centimetres of this solution into a vein. Collect the urine eliminated before and after the sugar injection. Test both with Fehling's solution for reducing substances. Collect samples of the urine every ten minutes after the beginning of the sugar injection. When does the sugar first appear in the urine? When does it cease to appear in the urine?

5. Intravenous Injection of Albumin.—Test the urine for albumin. If there is none present, inject, into a vein, ten to fifteen cubic centimetres of a 1-per-cent solution of egg albumin in physiological salt solution. Examine the urine for albumin at intervals of ten minutes.

6. Intravenous Injection of Peptone.—After the disappearance of the albumin from the urine, prepare a 2-per-cent peptone solution. Inject 10 c.c. of this solution into the vein. Collect the urine for ten minutes or until enough has been eliminated for testing.

Saturate the urine with ammonium sulphate. This precipitates mucin, albumin, and urates. Filter and test the filtrate for peptones by means of the biuret reaction.

Do peptones occur, normally, in the blood stream? If not, what becomes of the peptones that are absorbed from the gastric and intestinal mucous membranes? Is peptone ever found as an abnormal constituent of the urine? Under what pathological conditions may peptonuria occur?

7. Effect of Peptone on the Coagulation of the Blood.—Isolate and introduce a cannula into one carotid artery. Open the clamp on the artery and collect 10 or 15 c.c. of blood in a small test tube. Note the time taken for solidification of the shed blood.

Now inject 15 to 20 c.c. of the peptone solution into the vein. In three or four minutes open the artery clamp again; allow 2 or 3 c.c. to escape, and then collect in a small test tube 10 to 15 c.c. of blood. Compare the coagulability of this second sample with that of the first portion of blood shed. What is the effect of peptone upon the coagulability of the blood?

CHAPTER X.

SENSATION.

An organism is brought into relation with its environment through its irritability to external stimuli. This property, of irritability, is common to all protoplasm. As the organism increases in complexity from single-celled individuals to individuals consisting of groups of cells, this property of irritability or sensation becomes differentiated into a variety of sensations, depending upon the part of the external surface or special end-organ stimulated and the nature of the stimulus.

Conscious sensation first occurs, so far as we know, in those animals provided with a nervous system and brain. The sensory impulse is conducted over nerve pathways to the sensory portions of the cerebral cortex, and there interpreted in terms of sensation and corresponding judgments formed.

All sensations occur as a result of some form of stimulus applied to the outer body envelope and its connection through afferent nerves with the centres of consciousness in the brain. For the reception and transmission of certain stimuli, the outer envelope has become markedly modified, as, for example, the receiving apparatus for audition and vision.

The localities for the reception of certain sensory impressions are limited to certain sharply defined areas. These include the end-organs of taste, smell, sight, and hearing. Others have a wide distribution over the entire cutaneous surface and, to a lesser degree, over the mucous surfaces. Such are the tactile sense, the sense of temperature, the pain sense, and the pressure sense. The so-called muscular sense also has a wide distribution.

All parts of the body are brought into relation with the central nervous system through afferent or centripetal nerves. Only part

of these sensations, however, are commonly brought into the realm of consciousness. The majority of such impulses, from the viscera, for example, are either lost, through diffusion in the subsidiary parts of the nervous system, or are transferred, as corresponding efferent impulses, to complete the formation of reflex arcs.

When such impulses become abnormally intense, so as to overcome the resistance in the longer nerve pathways sufficiently to reach the realm of cerebral consciousness, the subjective sensations are either vague and indefinable, other than as a feeling of discomfort or pain somewhere in the region involved, or they are referred, as pain, to some part of the cutaneous surface whose afferent nerve distribution corresponds to the same cord segment as the efferent nerve distribution of the visceral area involved. Such reference of a sensory impression occurs, probably, for the reason that the sensorium is in the habit of receiving impulses from the skin area and not from the visceral area; and where the same terminal neurons transmit the impressions from the two sources, the sensation is referred to the area from which the impulses more usually come.

The nature of the conscious impression depends, not so much upon the character of the stimulus applied, as upon the peripheral area stimulated, the afferent nerve involved, and the brain area to which the impulse goes. Thus, a stimulation of the optic nerve, whether it be mechanical, electrical, or through the impact of light waves upon the retina, causes a sensation of light; stimulation of the olfactory nerves gives a sensation of smell; and of the taste nerves, of taste.

The cutaneous surface itself has been mapped out into areas or spots which are irritable to stimuli of various kinds. Thus, there are spots which respond to stimuli by a tactile sensation, others which are irritable to heat, others to cold, and others to stimuli which give a sensation of pain, independent of temperature or tactile sensation.

Quantitative Relation between Stimulus and Sensation.—In order that a stimulus may be effective in producing a sensation, its intensity must exceed a certain minimum value. This minimum

is sometimes spoken of as the threshold value of the stimulus. This threshold value is a variable quantity, varying for different individuals and for the same individual at different times. It depends partly upon the condition of the end-organ and the overlying integument, and partly upon the receptivity of the sensory cerebral area involved.

If the intensity of the stimulus is increased progressively above the threshold value, the intensity of the sensation increases also, up to a certain maximum, beyond which an increase in the strength of the stimulus produces no further increase in the intensity of the sensation. This maximum occurs with comparatively weak stimuli. The range of sensory variation is, therefore, not large. Between the maximum and minimum a variation in stimulus is accompanied by a variation in sensation. This variation cannot be measured by the subject of the sensation. He can tell that one stimulus is stronger or weaker than another, but not how much stronger or how much weaker.

An increase of the stimulus above the maximum of sensory interpretation very rapidly fatigues the sense organ. Even with weak stimuli, the sensory apparatus rapidly tires.

Weber's Law.—E. H. Weber, the first to make systematic observations along these lines (1831), formulated the following conclusion, which has since been known as Weber's law: "An increase in a stimulus sufficient to call forth a conscious increase in the sensation must always bear the same ratio to the original strength of stimulus to which it is added."

For example, if to a weight of 1 it is necessary to add a weight $\frac{1}{3}$ in order that the subject of the experiment may detect a difference, then, if a weight of 10 is used, the added increment necessary to produce an increase of sensation will be 10 divided by 3.

I. Cutaneous Sensation.

1. Tactile Sense.—To map out the touch-spots in a certain region of skin, some form of instrument, known as an æsthesiometer, is used. A simple form of æsthesiometer is made by fasten-

ing a hair at right angles to the end of a wooden handle by means of a bit of sealing-wax. If this hair is pressed, perpendicularly, against the skin, it will exert a certain pressure, depending upon the thickness and character of the hair. This pressure can be determined, for any hair, by pressing it against one scale pan of a balance and finding the largest weight that can be lifted in this way.

(*a*) Prepare a number of hair æsthesiometers, using hairs of different lengths and thicknesses and estimating their pressure values.

(*b*) Gently touch the end of a hair on the back of the hand. Note the sensitiveness of the hair as a touch organ. The end organs of touch are arranged in radiating lines about the roots of the hairs. The hairs act as levers, the long arm projecting above the skin surface and the short arm making pressure against the nerve endings. In this connection consider the so-called touch hairs of the cat and other animals.

(*c*) Shave the skin of the back of the hand, and, starting at the hair follicle, map out the touch-spots in an area of 2 sq. cm. Start with a test hair of least pressure and increase the pressure until sensation is produced. The subject of the experiment should be blindfolded and instructed to say yes immediately upon feeling the application of the test object.

Record the threshold value of the stimulus needed to produce sensation in this region. Record, also, the number of touch-spots present in the area of skin tested.

What is the arrangement of the touch-spots in relation to the hair follicle?

(*d*) Shave the skin of the back of the leg and map out the touch-spots and determine the threshold value of the stimulus, in the same way as was done for the skin of the back of the hand.

(*e*) Repeat the experiment for the skin of the abdomen, near the median line and some distance from the median line.

(*f*) Test the touch sensation of the skin of the back, over the shoulder.

(*g*) Map out the touch-spots of the cheek, starting near the lobe of the ear and proceeding to the angle of the mouth. In what part of this area are the touch-spots most numerous?

(*h*) Test the mucous membrane of the upper and lower lips in the same way.

(*i*) Test the palmar surfaces of the finger tips; of the hand.

How do the threshold values of the efficient stimuli, for the various regions tested, compare? Is there any relation between the number of touch-spots and the mobility of the regions tested?

(*j*) Repeat experiments (*a*) to (*i*), using, instead of the test hairs, a series of weights of the same surface area (about 4 sq. mm.). Begin with a weight of 0.0005 gm. and increase until the sensation is obtained. How do the threshold values compare with those obtained with the test hair? In the first series of experiments, single touch-spots were stimulated. In the second series, a number of touch-spots were stimulated simultaneously, the number varying with the region of skin to which the stimulus was applied.

The efficacy of any particular stimulus will depend, to a large extent, upon the number of touch-spots in the area stimulated, the proximity of the end organs to the skin surface, and the deformation of the skin effected by the stimulus. The temperature of the weights employed should be approximately that of the skin.

(*k*) Take a number of pieces of flat cork, cut into strips about 3 cm. long, 1 cm. wide, and 1 cm. thick, and pass blunted needles through the ends of the strips so that the distance between the needle-points of the different pairs varies from 1 to 25 mm.

With a blindfolded subject, test the ability of the skin of various regions to detect the application of the different pairs of needles as two separate stimuli. The subject should answer, immediately upon the application of the stimulus, one or two, as the sensation is that of one point or two points.

In this way test the sensitiveness of the skin of the finger-tips, the back of the hand, the shoulder-blade, the forehead, the cheek, the lips, the tip of the tongue, the skin of the thigh in its long axis, the skin of the thigh in its short axis.

In which of these areas are the two points of the test needles distinguished as separate, when brought nearest together? Why?

In which axis of a limb are the two points of the test object most readily distinguished? Explain.

Is there any after-sensation in any of the skin areas stimulated?

(1) Stimulation of the touch-spots, through pressure, occurs as a consequence of deformation of the skin. If a constant pressure is applied to all parts of a skin surface equally, there is no sensation of touch. This may be accomplished by immersing the hand in a vessel of water at the same temperature as that of the skin. So long as the hand remains quiet and the water is still, there is no sensation of touch, except at the junction of air and water at the surface of the liquid.

2. Temperature Sense.—Temperature sensation is of two kinds, sensation of cold and sensation of heat. There are, apparently, separate sets of nerve fibres and endings for these two sensations, since certain skin areas are irritable to cold objects but not to warm, and others are irritable to objects warmer than the skin but not to cold stimuli.

(*a*) These areas may be mapped out as so-called warm and cold spots by a method similar to that used in mapping out the touch-spots. Take a metal cannula, drawn to a fine point—a small artery cannula may be used—and run hot water through it for a time. Choose an area of skin, about 4 sq. cm. on the back of the hand, for testing. Bring the tip of the cannula sufficiently near the skin to obtain the sensation of heat without mixing this with the touch sense. Mark those points where the heat sensation occurs, with a fine-pointed colored pencil.

(*b*) Go over the same area of skin as in (*a*), running cold water through the cannula instead of hot as before. Mark the cold spots with a fine pencil of another color than that employed in marking the warm spots.

(*c*) A rough estimate of the temperature sense, in various skin areas of the body, may be made by filling two test tubes of small calibre with hot and cold water, respectively. These are applied,

alternately, to the same skin areas, and the sensation experienced is recorded and compared with that obtained in other skin areas.

(*d*) With a pair of blunt-pointed dividers, determine the nearest distance between the points at which they are detected as two. Now warm the points and repeat the experiment. Is the distance increased or decreased at which the points are separately felt by the skin?

CHAPTER XI.

VISION.

I. Dissection of the Eye.

1. Appendages.—(*a*) Examine the specimen before you, tracing out the *ocular* and *palpebral conjunctiva*, noting the *plica semilunaris* and the *caruncula*. How do the latter compare in relative size with the human structures? Locate and describe the *puncta lachrymalia* and the openings of the *lachrymal ducts*. How many are there? Is your specimen a right or left eye?

(*b*) Observe carefully the appendages, locating the *tarsal cartilages*, *Meibomian*, *sebaceous*, and *lachrymal* glands. Observe the *recti* and *oblique* muscles and their actions on the eyeball. Observe the entrance of the optic nerve.

2. By pinning down the flaps of the conjunctiva, fix the eyeball to the board, the cornea downward. Then dissect out the four recti and two oblique muscles, observing the capsule of Tenon.

Without injuring important vessels and nerves, remove the heavy retractor muscle. Locate and describe the *venæ vorticosæ*. How many are there? Find the *anterior ciliary arteries*. How many are there? What structures do they supply? Find the two *long ciliary arteries*, the *short posterior ciliary arteries*, and the *ciliary nerves*.

3. Eyeball.—(*a*) Fix the eyeball to the board, cornea up, pinning down the dissected muscles as guys. After having observed the cornea remove it with heavy scissors, near the corneo-scleral margin.

(*b*) Through the opening thus made, examine the iris. Where is the posterior chamber?

(*c*) Holding the margin of the cornea with strong forceps, dissect

the sclerotic coat free from the choroid, for about 3 mm. posterior to the angle of the anterior chamber. Between the insertions of the recti muscles, locate four points on the margin from which incisions may be made antero-posteriorly. From these points, make the incision posteriorly as far as the equator of the eyeball. Dissect each flap free from the underlying choroid. After having removed the pins fixing the recti muscles, draw the flaps back and fix. Observe the iridal and ciliary portions of the choroid.

(*d*) With a fine forceps, grasp the margin of the iris and with small scissors cut out a sector with the ciliary body as a base. Study the *posterior chamber*, *suspensory ligament*, and the anterior surface of the ciliary body.

(*e*) Make a circular incision with small scissors, severing choroid and retina at about the line of the ora serrata. Lift off from the vitreous humor the whole ciliary apparatus, placing it upon a plate, anterior surface downward. Observe the posterior aspect of the ciliary body. Describe the lens carefully, making a cross-section. Can you discern its capsule?

(*f*) Observe the retina, as seen through the vitreous, locating the entrance of the optic nerve. Can you locate the fovea centralis?

II. Physiological Optics.

Light is propagated from a luminous point in every plane and in every direction, in straight lines. These lines of direction are called *rays*. Rays travel with the same rapidity so long as they remain in the same medium; the denser the medium the slower the passage of light through it. The divergence of the rays of light is proportionate to the distance from which they come.

Rays of light proceeding from infinity are parallel. In dealing with rays of light which enter the eye, it will be sufficiently accurate to consider them parallel when they proceed from a point more than six metres distant.

A ray of light, meeting with a body, may be absorbed, reflected, or, if the body is transparent, refracted. In dealing with the eye, it is necessary to consider only the latter.

1. Refraction.—A ray of light passing through one transparent medium into another of different density is bent or refracted, unless the ray falls perpendicular to the surface of the denser medium. The ray is spoken of as *incident* before entering the second medium, and *emergent* after leaving it.

Upon entering a denser medium, the ray is refracted toward the perpendicular and from the perpendicular upon entering a rarer one. Reflection accompanies refraction, the ray dividing at the point of incidence.

THE INDEX OF REFRACTION is the relative resistance of a substance to the passage of light. Air is taken as a standard and is called 1. The index of refraction of water is 1.3, of glass, 1.5. The diamond has the highest refractive index, which is 2.4.

LENSES.—A lens is a transparent substance, usually glass, bounded by two curved surfaces, or by one plane and one curved surface.

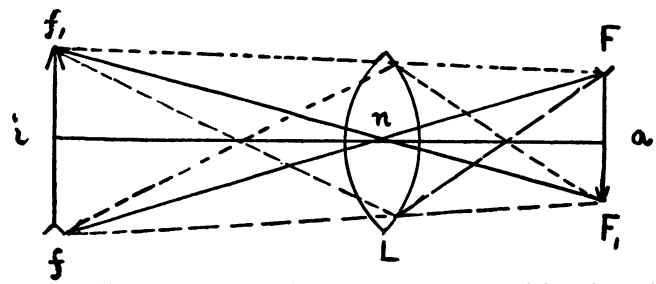

FIG. 40.—*a*, Object; *i*, image; *n*, nodal point; *L*, lens; *F*, *F*,, and *f*, *f*,, conjugate foci.

It may be regarded as a series of prisms. In a convex lens the bases are directed toward the centre, and in a concave lens the bases are directed away from the centre. Rays of light passing through a convex lens are made to converge. Those passing through a concave lens are made to diverge.

The point to which rays converge, after passing through a convex lens, is its *focus*. The *principal focus* of a convex lens is its focus for parallel rays.

When rays of light diverge from any point nearer than infinity, they are brought to a focus at a point beyond the principal focus.

The point from which they diverge and the point to which they converge are called conjugate foci. As one approaches the lens, the other recedes, and *vice versa*.

By means of a candle, determine the principal focus of the convex lens before you.

The foci of concave lenses for parallel or divergent rays are

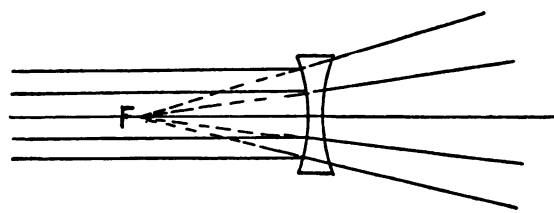

FIG. 41.—Described in text.

virtual or negative. They are the points from which rays seem to diverge after passing through the lens (see Fig. 41, *F*).

Parallel rays of light, falling upon a concave lens, are diverged. If these rays were traced backward, they would seem to diverge from a point nearer the lens (see Fig. 41, *F*).

The conjugate foci of concave lenses are also virtual and found in a similar manner. Find them.

Formation of images. The image of an object is the collection of the foci of its principal points.

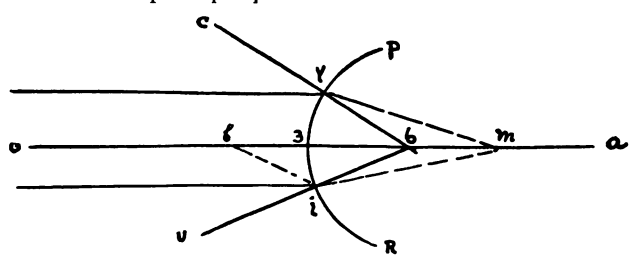

FIG. 42.—Described in text.

SIMPLE DIOPTRIC SYSTEM.—The simplest form of a dioptric apparatus consists of two media of different refractive indices,

separated by a spherical surface. In such an apparatus, the optical properties depend upon the curvature of the surface and the refractive power of the media. Such an apparatus is shown in the accompanying figure (Fig. 42). The line *pr* represents a curved surface separating media of different refractive power, the lens being on the left. The line *oa*, falling perpendicularly upon the surface at 3, passes through the centre of the sphere, 6. The line *o-a* is the optical axis. All the lines that cut the surface normally, such as *o-a*, *c-y*, and *u-i*, undergo no refraction and, continuing in straight lines, cross at 6, which is the nodal point. All of the rays are refracted. All rays, parallel to the optical axis, passing through the lens will be bent so as to meet at *m*, which is the posterior principal focus. The anterior principal focus is at *b*, in the first medium and in front of the lens. Rays of light, such as *b-i-t*, passing from it, are so refracted that they become parallel to the optic axis. The principal point is the point where the optic axis cuts the surface. The posterior, anterior, nodal, and principal points are the cardinal points of an optical system.

THE EYE AS AN OPTICAL INSTRUMENT.—Having reviewed the general optical principles concerning the refraction of light and the formation of images by convex lenses, we now come to the eye as an optical instrument. Rays of light, as they enter the eye encounter not one refracting medium as in the simple dioptric system, but five, namely:

Tears,
Cornea,
Aqueous humor,
Lens,
Vitreous humor.

The indices of refraction of these various media are such that parallel rays of light, entering a normal eye, are brought to a focus upon the retina. For the sake of simplicity, they may be looked upon as equal to a convex lens of about twenty-three millimetres focus. However, a ray of light falling upon the cornea does not follow the same simple direction it would, were it to pass through a

single medium. Instead, the eye must be regarded as a compound refracting system, composed of a spherical surface and a biconvex lens.

The cardinal points are

Two principal points,

Two nodal points,

Two principal foci.

In the diagram, Fig. 43, the cardinal points are shown all upon the optic axis, *f-a*. At *b*, two principal points, situated so

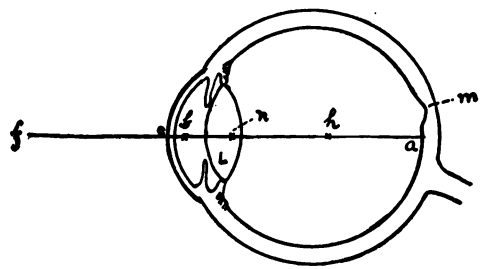

FIG. 43.—Described in text.

close together in the anterior chamber that they may be regarded as one. At *f* is the first principal focus, and at *a* the second. The nodal points correspond nearly to the optical centre of the refractive system. Rays passing through these points are not refracted. They are situated about 7 mm. behind the cornea.

THE FORMATION OF RETINAL IMAGES. A luminous point placed above the principal axis has its image formed upon the retina below this axis, and *vice versa*. Replace these points by an object and the same thing occurs. The retinal image is, as it were, a mosaic, composed of innumerable foci of the object.

Construct a simple diagram of the human eye, showing the formation of an image, say an arrow or a candle, upon the retina. Is the image erect or inverted? If inverted, why do we see it erect?

The human eye has aptly been compared to a camera, the refracting media representing the camera lenses, and the retina its sensitive plate.

Adaptation of the Eye for Distance.

In the camera, however, it is necessary to adjust the instrument by backward and forward movements of the lenses. In the eye, this adjustment is brought about by changes in convexity of the lens, or *accommodation*. Accommodation is, therefore, the functional adaptation of the eye to distance.

If the entire optical apparatus of the eye were rigid and fixed, how could objects at various distances be seen clearly? Explain what takes place when the eye accommodates.

Take a sharp-pointed pencil in each hand. With one eye closed, hold the points in a direct line of vision before the other eye—one, about twenty centimetres distant, and the other a full arm's length. Focus on the nearer pencil. Is the image of the distant one clear? Focus upon the farther pencil. Is the image of the nearer one clear?

The near point, or *punctum proximum*, is the nearest point to the eye to which objects may be brought and still be seen clearly. It averages about 12 cm. At this point the accommodation is most active.

Determine your own near point.

The far point, or *punctum remotum*, is the farthest point at which objects may be seen clearly by the normal eye.

The *range of accommodation* is the difference between the punctum proximum and the punctum remotum.

Determine your own range of accommodation.

Adaptation of the Eye for Direction.

As the eye can functionally adjust itself to distance, it can also change the direction of its visual axis from one object to another, or can follow objects moving within its field of vision.

Two students may work together, one as observer and the other as subject.

(*a*) Monocular Fixation.—The observer and subject being seated opposite each other, let the subject close or screen one eye.

(1) Hold any small object directly in front of the subject and have him fix his eye upon it constantly. Move the object quickly toward the subject's left and notice the immediate fixation of the object in its new position. What muscles are brought into action, in this movement? (2) Move the object quickly to the right, upward, downward, and diagonally, noticing the immediate fixation in all the fields. What muscles are brought into action in each position, and are all movements equally rapid? (3) Bring the object exactly in front about one metre distance and note the range of lateral movement without causing any appreciable change in the visual axis. (4) Bring the object to the central position and move it very slowly outward in various directions and observe whether the changes of direction of the visual axes are equally slow and regular.

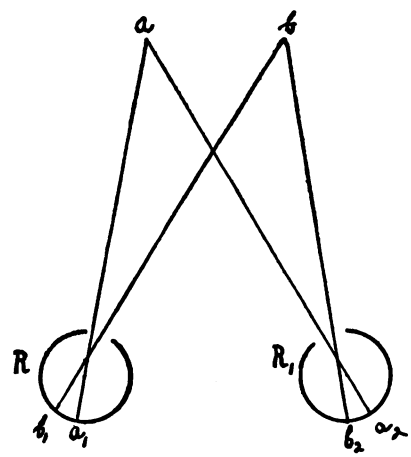

FIG. 44.—a, b, Two objects, the images of which (a_1, b_1 and a_2, b_2) fall on corresponding parts of both retinæ, R and R_1.

(*b*) BINOCULAR FIXATION.—*Convergence.* — It was probably noticed during the above exercises that, though one eye was screened, it shared in all movements with its fellow. With both eyes open, let the subject fix a small object, held about one metre distant. Let the observer move the object slowly in all fields, downward, upward, laterally, and around, observing the perfect continuous fixation with both eyes.

What muscles or pairs of muscles are involved in the movements in the different directions? If any variations are noticed in the subjects examined, describe them.

STEREOSCOPIC VISION.—*Binocular Single Vision.*—By this is

meant the union, in one single impression, of images received simultaneously on both retinæ. The external ocular muscles maintain the visual axes parallel, so that impressions of an object fall on correspondingly identical points of both retinæ (see Fig. 44).

What happens when paralysis of one ocular muscle destroys balance? Show by diagram.

CONVERGENCE.—Let the subject fix his vision upon an object within an arm's length, and then upon some object more than six metres distant but in the same line of vision.

What change takes place in relation of the visual axes to each other?

Hold an object one metre distant, directly in front of the subject. Move it directly toward the subject's eyes. Note the convergence of the visual axes.

What change takes place in the pupil? What muscles are involved in the act? Recall the innervation of the pupil and the internal recti.

OPTICAL DEFECTS.

The eye is not a perfect optical instrument, since it is not exactly centred and possesses in small degree chromatic and spherical aberration.

By chromatic aberration is meant that different rays of the spectrum are bent to different degrees. For instance, violet rays, which are more refrangible than red, have their focus near to the lens. In the manufacture of optical instruments, this is overcome by combining a convex with a plano-concave lens. Practically the same arrangement exists in the eye, and this, combined with the rapid accommodative ability of the lens, makes chromatic aberration a negligible quantity.

By spherical aberration is meant that rays of light which traverse the periphery of a lens are brought to a focus sooner than those which pass nearer the centre. The iris corrects this defect by acting as a diaphragm, shutting off the peripheral rays.

VISION.

Miscellaneous Experiments.

Blind Spot.—On a white card make a small black cross, and about 8 cm. away, in a horizontal direction, draw a black dot. Looking intently at the dot, one eye having been screened, hold the card about 25 cm. from the eye and then move it slowly toward the eye. At what distance does the cross disappear? Why?

Imperfect Visual Judgments.—Draw one line (horizontal), 6 cm. long, and one perpendicular to the same length but not joining. Which line appears the longer and why?

Make three black dots on the same imaginary horizontal line and equidistant from each other. Mark them x, y, and z. Connect x and y with a series of equidistant dots of the same size. Which appear farther apart, x and y or y and z?

Draw two horizontal lines 5 cm. long. On the upper one make arrow-heads pointing toward each other. On the lower make arrow-heads pointing away from each other. Which line appears the longer?

Draw three parallel lines. On the upper one draw a series of short, parallel intersecting lines, cutting the longer line at an angle. Do the same with the middle line, except to make the angle of intersection equal and opposite to that of the first series. Prepare the lower line the same as the upper line. Do the lines still appear parallel?

Sanson-Purkinje Images.—Darken the room. Hold a lighted candle a little to one side and in front of the subject's eye. The observer, looking at the eye from the other side, sees three images of the flame. The first and brightest is a small, erect image formed by the anterior convex surface of the cornea. The second, larger and less distinct, is formed by the anterior convex surface of the lens. The third, smaller, inverted, and indistinct, is formed by the posterior surface of the lens. Let the subject accommodate for a near object. Describe the change in relation that takes place in the size and clearness of the second image and its proximity to the first.

Duration of Impressions.—On a circular white disc, midway between the periphery and centre, fix a small black oblong disc. Rotate it rapidly. Note that a ring of gray appears on the black, showing that retinal impressions are of a certain duration.

Inversion of Shadows.—Make three pinholes in a card, close together and arranged in a small triangle. Hold the card about 12 to 15 cm. from the right eye. Look through the holes at a bright light. Close the left eye and hold a pin in front of the right eye, so that it just touches the lashes. Note that an inverted image of the pin will be seen in each hole. Retinal images are inverted. Shadows are erect. Therefore the latter, upon being projected outward into space, are seen inverted.

Normal Vision.

Examination of Distant Vision.—The sense of sight consists of (1) form sense (acuity), (2) light sense, (3) color sense.

The acuteness of direct vision is measured by means of letters, sized to certain definite standards. Those devised by Snellen are in most common use. Snellen determined the normal acuteness of vision to be the power of distinguishing letters subtending the visual angle of 5′. The letters are formed of strokes whose width is one-fifth the size of each letter, hence they are seen under an angle of 1′. The openings in the letters, and the spaces between the contiguous strokes, are made to conform, as nearly as possible, to the same angle.

The relation of the size of the letter to the distance at which it should be discerned by the normal eye is expressed by twice the tangent of half the angle of 5′, or, 0.001425. The size of the letter, the perception of which constitutes normal vision at a given distance, may be obtained by multiplying the distance by 0.001425. On this, the standard letters of measuring visual acuity have been built up.

Practical experience, however, has shown that letters constructed under the angle of 5′ do not always give the best visual acuity of

which the subject is capable; so that figures constructed on the 4′ basis are gradually coming into use.

For recording visual acuity, the formula $V = \frac{d}{D}$ is used, V standing for vision; d, for the distance of the subject from the test type; D, the distance from which it should be read.

In practice, the acuteness of vision is found by determining the smallest type the subject can read at six metres. Normal vision is represented by the symbol $\frac{6}{6}$. That is to say, that at six metres the subject reads the test line marked 6. If, at this distance, the subject can only read the line marked 12, his visual record would be $\frac{6}{12}$, and so on.

In instances where the vision of the subject is lowered to the extent that he cannot see even the largest test types, vision is tested by the ability to count fingers at varying distances. For example, V = fingers, 3 m. If vision is still lower, V = shadows or light.

Examination of Near Vision.—This includes the ability of the subject to read print. That is the condition of accommodation. The test types are those of von Jaeger and Snellen.

Exercise.—Test the visual acuity of each other, recording your findings, both for distant and near.

Light sense is the power possessed by the retina of appreciating variations in the intensity of light. It is measured by the photometer, which consists, essentially, of an apparatus by which the intensity of two sources of light may be compared.

Color Sense.—This is the power the retina has of distinguishing or perceiving colors, or the impression resulting from the impact of light rays having different refrangibilities.

Holmgren's test consists of testing the power of a subject to match various colored yarns. Three large test skeins, namely, (1) light, pure green, (2) rose-purple, (3) red, are given the patient, and also smaller skeins, comprising various shades and tints of each color. He is requested to pick out the colors similar to his original three skeins. If, for example, he is red-blind, he will not

see the red in the purple or related colors, but will classify these with the blues, while the reds will be confused with the greens. Make the test.

Abnormal Vision.

Emmetropia is that condition in which the eye, in a state of rest, focusses parallel rays of light exactly upon the retina.

Ametropia is any deviation from the condition of emmetropia.

Hypermetropia or hyperopia (far-sightedness) is that form of ametropia in which the eye, when at rest, focusses parallel rays of light behind the retina. It is corrected by placing before the eye a convex lens. Why? Make a diagram.

Myopia (near-sightedness) is that form of ametropia in which the eye, when at rest, focusses parallel rays of light in front of the retina. It is corrected by placing before the eye a concave lens. Why? Make a diagram.

Astigmatism is that form of ametropia in which the eye, when at rest, does not focus parallel rays of light upon any one spot. Or, in other words, rays of light, coming through different meridians of the eye, come to a focus in different planes, perpendicular to the visual axis.

There are various kinds of astigmatism, depending upon the focal points of the different meridians.

(*a*) *Regular astigmatism* is that form in which the rays of light, passing through each meridian, are equally refracted in all parts of the meridian, but the refraction of at least two meridians is dissimilar.

(*b*) *Irregular astigmatism* is that form in which the various parts of the same meridian refract rays unequally.

(*c*) *Simple hyperopic astigmatism* is that form in which the rays passing through one principal meridian are brought to a focus on the retina, while those passing through the other principal meridian are focussed at a point back of the retina.

(*d*) *Compound hyperopic astigmatism* is that form in which the

rays from both meridians are focussed back of the retina, but at two different points.

(*e*) *Simple myopic astigmatism* is that form in which one meridian refracts rays to a point in front of the retina, and the other principal meridian focusses rays upon the retina.

(*f*) *Compound hyperopic astigmatism* is that form in which rays passing through both principal meridians are brought to a focus at different points in front of the retina.

(*g*) *Mixed astigmatism* is that form in which one meridian fo-

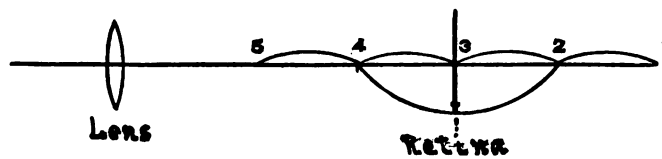

FIG. 45.—Described in text.

cusses rays in front of the retina, and the other focusses them back of the retina.

In simple hyperopic astigmatism the rays are focussed at 2 and 3 (see Fig. 45).

In compound hyperopic astigmatism the rays are focussed at 1 and 2.

In simple myopic astigmatism the rays are focussed at 3 and 4.

In compound myopic astigmatism the rays are focussed at 4 and 5.

In mixed astigmatism the rays are focussed at 2 and 4.

(*h*) *Presbyopia* is loss of accommodative power due to sclerosing of the crystalline lens. Although the process commences during the first year of life, the lens does not lose enough of its elasticity to interfere with near vision until about the age of forty. It is corrected by placing before the eye a convex lens. Why? Make a diagram.

CORRECTION OF REFRACTIVE DEFECTS.

The Numbering of Lenses.—Lenses are measured according to their refractive power. A lens whose focal distance is one metre

is taken as the unit of measure. It is numbered 1, and is called one diopter (D). The refractive power of a lens is the inverse of its focal distance. Hence a lens of 2 diopters has a focal distance of 0.50. What is the focal distance of a lens of 4 D? Given a lens whose focal distance is 2 metres, what is its number?

Parallel rays of light, passing through a convex lens, are made to converge. Parallel rays of light, passing through a concave lens, are made to diverge.

A cylindric lens is a lens, one or both surfaces of which are segments of a cylinder. Rays of light, passing through it in a plane parallel to its axis, are not bent. Rays passing in a plane perpendicular to its axis, converge or diverge, according as to whether the cylinder is concave or convex.

Lenses are designated plus (+) if they are convex, and minus (—) if concave.

What forms of ametropia would cylinders correct?

Ophthalmoscopy.

The ophthalmoscope is, in its simplest form, a mirror with a hole in it. The first instrument of Helmholtz, in fact, consisted of three thin plates of glass, fastened together and mounted in a frame, at an angle of 56°. The whole object of the instrument is to illuminate the ocular fundus by reflected rays of light and permit the observer to inspect the illuminated area. All patterns are useful. The patterns of Loring and Morton are most popular.

Use.—Not all the light entering the pupils is absorbed by the pigmentary layer of the choroid. A certain amount returns from the eye. If, therefore, the observer's eye is placed in the same position as the source of illumination, or directly behind it, the interior of the eye becomes visible. This is the principle of the ophthalmoscope. The mirror, which gathers rays of light from a luminous point, becomes a secondary source of light which is projected into the pupil.

Methods.—There are two, direct and indirect. In the direct, the examiner places his eye close to that of the patient and looks

directly upon the enlarged and upright details of the fundus. In the indirect, the subject is at an arm's length, and a convex lens is placed between the subject's eye and the examiner's mirror. The image, as obtained, is inverted and aërial. The two methods differ, practically, in that the direct image is larger and erect, in a small field, while the indirect image is smaller and inverted, but in a larger field. The two methods are explained in the accompanying figures (46 and 47).

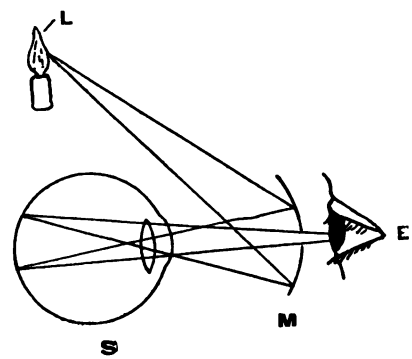

FIG. 46. — Use of Ophthalmoscope. Direct method. *S*, eye of subject; *E*, eye of observer; *M*, mirror; *L*, source of light.

Examine the fundus, both by the direct and indirect method, and make an outline drawing showing the disc and retinal vessels in each case.

PERIMETRY.

In contradistinction to visual acuity which is limited to the macula, the function of sight performed by the other parts of the retina is called indirect vision.

The limits of the field of vision are best obtained by an instrument, the perimeter, but a fairly accurate map of a field, not larger than 45°, may be obtained on a blackboard.

Exercise.—In the centre of the blackboard, in the line of direct vision, locate a dot, the point of fixation. Draw from the dot, as a centre, a series of circles whose distance from each other shall represent an angular distance of 10°.

Now draw the meridians which will divide each quadrant into at least three subdivisions.

A wooden guide, twenty centimetres long, should be provided

to regulate the distance of the subject's eyes from the point of fixation. The subject, with one eye screened, places himself directly in front of the point of fixation and twenty centimetres from it. Make a test object out of a piece of white paper, one centimetre

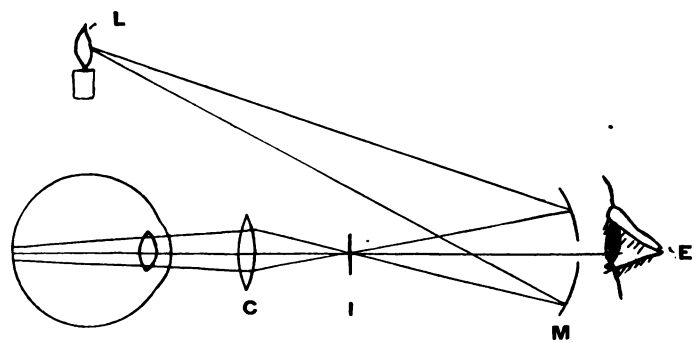

FIG. 47.—Use of Ophthalmoscope. Indirect method. *S*, eye of subject; *E*, eye of observer; *M*, mirror; *L*, source of light; *C*, convex lens; *I*, image of fundus.

square, and affix it to a black handle. Let the operator move the test object along one meridian, say the horizontal, from the periphery toward the point of fixation. As soon as the subject sees the test object, make a chalk mark on the meridian, denoting the place where it is first seen. In like manner go over at least eight of the meridians. Join the points so obtained with a line, and the result is the approximate field for white.

In the same manner map out the field for blue and red.

Which field is the largest, that for white, for blue, or for red? Which is the smallest?

DRUGS ACTING LOCALLY ON THE EYE.

Those acting directly upon the eye are divided into (1) mydriatics (dilators of the pupil), such as atropine, homatropine, cocaine, scopolamine, etc.

(2) Myotics (pupil contractors), such as eserine and pilocarpine.

(3) Cycloplegics (paralysants of the ciliary muscles), such as atropine, homatropine, scopolamine, etc.

(4) Anæsthetics (local), such as cocaine, eucaine, holocaine.

Of the mydriatics, the first four are in most common use. They differ from one another in intensity and duration of effect, in the order named. The mydriatics, as a rule, increase the intraocular tension.

Exercise.—Instil a drop of homatropine into a subject's eye. What effect does it have on the pupil? Upon accommodation? Explain the action.

Instil a drop of eserine in the same eye. What effect is produced?

Instil a drop of cocaine into another subject's eye. Brush the cornea, lightly, with a wisp of cotton. Note the anæsthesia and the effect on the pupil. Which produces the more complete mydriasis, cocaine or homatropine?

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